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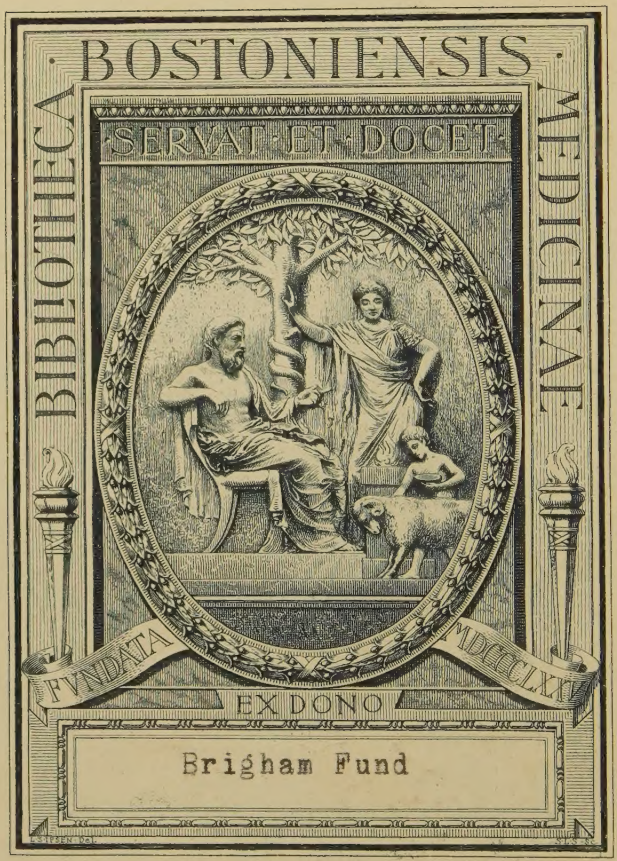

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ELEMENTARY BACTERIOLOGY

By

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and

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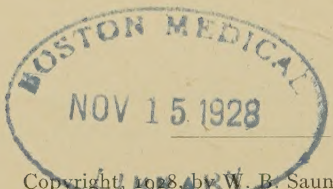

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PREFACE

Bacteria rule the world. Man is dependent on them from the day of his birth until the hour of his death. They are man's most useful servants and his most destructive master. It was they who first obtained a precarious foothold on the bleak primitive rock of early geological periods. Here they fed on the carbon dioxide of the air, the ammonia of the rain waters, and the traces of minerals which the water dissolved from the rocks. They acting in conjunction with physical and chemical agents eventually rendered the disintegrating rock a fit abode for higher plants. This paved the way for the higher animals and when they appeared bacteria settled on the skin and mucous membranes of their bodies. Most such bacteria perished, some became parasites while a few became progressive pathogens. These latter have become man's most destructive enemies and modern bacteriology deals to a considerable extent with their doings because there has been a never ending struggle between them and man to determine who shall possess the earth. They have defeated armies and destroyed cities. Nations which have been the glory of the world have gone down before them. They are no respectors of persons, nor are they governed by the edicts of rulers. However, at the command of modern science they are commencing to relinquish their hold, and some of earth's most fertile regions, where in the past the risk of death to the visitor was greater than a visit to the battle field, are being transformed into tropical health resorts. Still much remains to be done for the learning and the practicing of the laws of sanitation can prevent more suffering and save more lives than the abolition of war.

However, we should not lose sight of the fact that the great majority of all bacteria are innoxious and in many cases beneficial. Bacteria tear down the highly complex bodies of plants and animals from which the spark of life has departed, and salvage the parts so that they may be used again, thus restarting the elements on their wonderful constructive cycles. They help man in the

arts and industries, at first in a chance and haphazard way but as time went on man learned how to intelligently direct the action of the beneficial bacteria and also how to control the injurious microbes. This knowledge has resulted in a better world for man to live in, love in, rear his children in, and to die in at a riper age, because the knowledge gained is ever increasing man's expectancy of life.

In this book we hope is narrated in an elementary and interesting manner the doings of the beneficial and injurious microbes, and incidentally man's struggle to obtain mastery over his microscopic friends and foes. We have given briefly, but we hope not inadequately, information on classification and morphology requisite for an understanding of the important physiological actions of the interesting and important group of microörganisms known as bacteria. The meager consideration given to classification and morphology should not be taken to indicate that we depreciate their value but merely that we believe they should not be "ends but means" in an elementary course. Nor should the absence from this book of laboratory instructions be taken to indicate that laboratory work is not important. They are left for laboratory manuals.

The book has been written with the hope of furnishing a suitable text for courses in elementary bacteriology, yet it should prove of interest to nurses, home demonstrators, agricultural and home economic workers, and to that ever increasing multitude of readers who are turning from fiction to facts. For within the field of bacteriology one learns of curious, wonderful, marvelous and often beautiful specks of living protoplasm which have determined when, where, and how man may live. They have been more powerful than kings and potentates and have to a great extent moulded human history. In the subject are portrayed man's combat with invisible foes; combats which from the standpoint of heroism and determination rival military or even imaginary legends of antiquity, and as one reads one can remember that they are facts not fiction, and deal with the present not antiquity. It deals with a science in the making which today is calling for more Pasteurs, more Kochs, more Ehrlichs, more Lazears, and more Carrolls, who will conquer man's microscopic enemies and learn to guide his microscopic friends, for each bit of knowledge gained in this field carries man one step nearer the goal where he will be the master and not the slave of his environment.

To our many friends our hearty thanks are offered for the valuable encouragement and assistance given in the preparation of this book. We are especially happy to acknowledge the many valuable suggestions we have received from Dr. John A. Widtsoe, Dr. T. L. Martin, Dr. H. J. Frederick, Professor C. T. Hirst, and Fred Hammerly who have read parts or all of the manuscript. Nor do we forget the assistance rendered by J. Dudley Greaves in preparing many of the illustrations and to Claude Zobell, J. Dudley Greaves, and Florence D. Greaves for help in much of the routine work. We are also deeply grateful to Mrs. Blanche C. Pittman and Miss Maida Muir for their painstaking care in the preparation of the manuscript for the press.

J. E. G.
E. O. G.

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ELEMENTARY BACTERIOLOGY

CHAPTER I

INTRODUCTION

One autumn day I was seated in a cafeteria when a party of six entered. They served themselves and proceeded to a table nearby just vacated by another party. One of the members took from his pocket a handkerchief and brushed the crumbs from the table. Soon the party was enjoying their midday meal. I had known at college the young gentleman who had so nicely dusted off the table. He was a brilliant man, well trained, and had meritoriously gained his Ph.D. He had incidentally come into possession of T.B.

Some time later I stopped at a hotel with two other college men, both of whom had received their advanced degrees from leading institutions of the country. I noticed that each carried his tooth brush and conscientiously used it each night and morning. One of them placed his moistened brush on the edge of the washbasin while he searched for his tooth paste which when found was used with his polluted brush.

These two incidents, together with many others of similar nature, have often caused me to ask myself the question: In this day when health work has shifted from the environment to the individual and when each individual can do so much to prevent infection, are individuals prepared for life in our modern, intimate, and complex society without learning the a b c's of sanitation? The few fundamentals that the physician uses when visiting cases of communicable diseases and the same principles that have made it possible for the trained attendant to nurse infectious diseases without contracting them herself or conveying them to others can be learned and used by all.

Fundamentals.—Today we smile as we read van Helmont's formula for producing mice: "Place some dirty rags together with a few grains of wheat, or a piece of cheese, in a dark place

and in a few days they will be transformed into mice." The same philosopher's method of engendering scorpions is amusing to us: "Scoop out a hole in a brick, put into it some sweet basil. Lay a second brick upon the first so that the hole will be imperfectly covered. Expose the two bricks to the sun, and at the end of a few days the smell of the sweet basil, acting as a ferment, will change the herb into a real scorpion."

How many are there today who believe that disease germs breed in stagnant water, deserted lots, decaying vegetables, and other bad surroundings? What percentage of humanity today know that disease-producing microörganisms are always the descendants of other similar disease-producing microörganisms, and if these are kept away from the body of man that there never can develop within him an infectious disease be his physical condition ever so bad? These microörganisms always breed true, and it is just as possible to grow a crop of diphtheria germs, typhoid germs, or tuberculosis germs as it is to raise a crop of potatoes; and the bacteriologist would be just as surprised to reap a crop of diphtheria where he planted typhoid as the farmer would be to harvest potatoes where he had planted corn.

Special plants require special soil and definite conditions of temperature, moisture, and aëration for their growth. The medium in which the disease-producing organisms grow is with few exceptions limited to man and the lower animals; hence, when seeking these specific types of organisms for laboratory study, we turn to man and the things which he has handled.

The evidence is complete that the causative agents of tuberculosis, pneumonia, influenza, cerebrospinal meningitis, scarlet fever, typhus fever, small-pox, whooping cough, gonorrhea, syphilis, malaria, yellow fever, and sleeping sickness multiply only in the body of animals. It is also an established fact that the number reaching the body of one animal is no larger than the number that leave the body of another animal.

Diphtheria, which for so long has been considered a filth disease, that is, the germs were supposed to have a habitat outside of the body in various forms of dirt, is now known to be a purely contagious disease. The organism is more resistant than are some other disease producers, but there is as yet no evidence to show that it is propagated outside the body except occasionally in milk.

Whereas it is well known that water often gives rise to typhoid fever, it is also well established that typhoid bacteria rapidly

disappear from water and probably never live in water longer than fifteen or twenty days. There is no reason for believing that the germ ever multiplies in water. Hence, water requires a constant source of new infection from the body of a human individual to be at all dangerous, for it is evident that while the typhoid bacillus may live for some time in the soil there is no evidence that it can multiply in soil. This organism, then, along with many others, has its origin only in man. Therefore, man is man's greatest enemy so far as the production of disease is concerned.

From time immemorial vapors and emanations, gaseous or otherwise, have been considered to be frequent causes of disease. But with the growth of the subject of bacteriology it was found that bacteria were the real cause of disease. Since then, even among many educated persons, the favorite explanation of the transmission of disease has been that diseases are conveyed in the air. Experience has taught that even smallpox or measles can be housed in the same hospital or even in the same wards without danger provided the necessary precautions are taken to prevent the carrying of infection from one individual to another by the attendants.

This gives rise to the question: How are the various disease producing bacteria spread?

Some of them use the common housefly as their airship, others the flea, and still others chance a ride with the bedbug. At other times they find their way into food, or into clothing and are transported from place to place in this manner. Some are picked up with the particles of dust, but to the disease-producing organisms this method of travel is highly fatal, for when struck by the direct rays of the sun they are soon killed. Many could not make any long journey on dust particles even in the dark for the bodies of the organisms are about 90 per cent water, and if not clinging to some moist substance they soon perish.

Numerous disease-producing bacteria are found in the mouth of apparently healthy individuals, and it is a matter of little difficulty for these organisms to journey from the lips of one individual to the lips of another. The ordinary drinking cup is only one of the hundred and one things which come in contact with the mouths of many individuals every day, and serve as the means of transportation.

Then again, "our fingers are ten swabs which are continually picking up all sorts of dirt from door knobs, stair rails car straps,

counters, chairs, books, money, water closets, our own shoes and rubbers, and other things, the list of which can be extended almost indefinitely. Visible dirt gathers quickly. With the visible dirt is also gathered all too frequently some of the secretions from another person. Often these secretions contain the germs of tuberculosis, diphtheria, meningitis, or scarlet fever. Watch again how often the fingers go to the lips. Unfortunately these finger swabs, unlike the cotton swab of the physician, plant their germs not in the test tube but on human mucous membrane."

Hence, while the three R's may be fast slipping from the first place in our educational curriculum, the three F's—fingers, flies, and food—reign supreme in sanitation; and the individual who has these facts well grounded has a powerful weapon with which to protect himself and others.

We should laugh to scorn the individual who today would advocate keeping in the prime of condition, so that he might resist the bullets of the enemy. Yet, how many believe that general good health protects against infection? We need only use our eyes to become aware of the fact that the physically fit and robust fall prey to typhoid fever, smallpox, influenza, and most other infectious diseases as well as the weaklings.

One should keep in the best of physical condition, for it is not until then that life is really made worth living; but everyone must learn that disease-producing organisms must be kept away from his body if he wishes to avoid infection. This implies a knowledge of where these microörganisms grow, how they are carried from their place of growth, and how they enter the body. In some cases where it is impossible to keep them out of our bodies the situation becomes grave, unless we have within us the specific antidote that science has discovered against them. This is especially true in respect to smallpox, typhoid, diphtheria, tetanus, and a number of other diseases. Too often do we find even educated individuals opposing the use of these antidotes because of the danger they believe to be associated with their use when as a matter of fact the individual who is vaccinated or given an antitoxin is not endangering his life or limb as much as the person who takes a motor ride.

Facts in Disease Prevention.—We have then certain fundamentals in the prevention of disease that should be the common knowledge of every boy, or girl, man or woman.

(1) Microörganisms are the cause of the communicable diseases and these are the descendants of other similar microörganisms.

(2) The overwhelming majority of all microörganisms which cause disease in man multiply only in the body of man or the lower animals.

(3) Diseases are transmitted through direct contact or through the intervention of insects. In other words, the principal carriers of disease are fingers, flies, and food.

(4) A high state of bodily health should be the aim of all, but this alone will not confer immunity to the communicable diseases. Such immunity may often be conferred by the vaccines and antitoxins.

Moreover, it should be understood that there is an ethical side to the subject, for whereas the United States constitution guarantees to each of us life, liberty, and the pursuit of happiness it does not give us the right to infect another carelessly. All individuals are willing to wage a relentless war on flies, mosquitoes, and the lower animals, so that these will not carry disease to man, yet they are not willing to remain from the theater when suffering with a cold, to refrain, when feeling indisposed, from handling the food or milk to be used by others, to see that the milk they are selling to their neighbor, or feeding to their own children is free from disease germs.

The relentless war that has been waged against lower animals is causing cholera, plague, and yellow fever to disappear from the face of the earth, but no such success has attended the directly communicable diseases as some of these are on the increase. Yet our knowledge at the present time is sufficient to stamp out certain diseases that are on the increase if each would but learn that he is his brother's keeper.

What a glorious attainment it would be if everyone could have firmly rooted in his nature this slogan: I am building for myself and future generations; hence, the structure which I rear must be built on a firm foundation. For the cornerstone I shall use *Character*, for the stones *Health*, and for the cement *Education*. The building stones I wish free from blemishes and defects, and in order to keep them so I shall learn and live the laws of sanitation.

Can Be Learned by Young.—That these principles can be learned by the very young is shown by the following: A five-year-old boy, who had watched the growth of disease-producing bacteria in media and had seen the methods of destroying them, found a bell which he wished to present to his baby sister. His mother's attention was first directed to it when the boy attempted

to get the bell from the oven where it had baked for several hours so it would be a fit plaything for sister. When the baby sister was presented with the bell it had been sterilized as effectively as could have been done by the best physician.

When the baby sister became old enough to enter school a teacher stopped her father on the street one day and said: "We had a very amusing experience in school today. When the doctor was examining your little girl he asked her to open her mouth, but as his hand moved toward her mouth she closed it and opened it only when commanded to do so, closing it again every time the hand of the examiner moved toward it. This was repeated a number of times until between sobs she looked at the doctor and said, 'You can't put your finger in my mouth; it's got germs on it and they will make me sick.'" Not only can these principles be learned by the young, but they should be made an intimate part of their education. To be sure they are only children, but the children of today will be the future fathers and mothers and they in turn will nurse any communicable diseases that may be present within their families, and pass them on to others and to their children's children.

Deals with the Interesting.—There is no subject which has a wider appeal than bacteriology. If one is of a mathematical turn of mind he can apply his knowledge of geometrical progression to the problem of the multiplication of bacteria. If bacterial multiplication went on unchecked the descendants of one cell would in two days number 281,000,000,000. What would it be at the end of a month? What would be the weight in tons of the resulting mass when one cell weighed one-hundred millionth of a milligram? These are some of the problems that one might wish to solve.

If one loves the beautiful and the curious, nothing can be found that is more fascinating than the study of pigmented bacteria whose bodies contain the most remarkable varieties of colors. In this connection an explanation for many of the weird stories of ghosts and goblins can be found, particularly in the study of the light-producing fluorescent bacteria which often cause meat or decaying stumps to shine at night with an uncanny light.

If one is interested in stories of heroism, they are to be found in the history of bacteriology as in no other place in the annals of history. Should anyone doubt this let him read the life of Pasteur during the time he was studying rabies, the history of

the conquest of the plague, the story of the work of the Yellow Fever Commission which made possible the digging of the Panama Canal. Here one can follow men in their valiant struggles against an invisible foe. One can follow the dark and anxious hours of sorrow and suffering before the causes of the various diseases were found. Then one may read the inscriptions on the tablets such as the one erected to the memory of Dr. Lazear who gave his life in the fight against Yellow Fever, and which reads: "With more than the courage and devotion of the soldier, he risked and lost his life to show how a fearful pestilence is communicated and how its ravages may be prevented."

If one wishes to see examples of great determination these can be found in the lives of men like Ehrlich, who undertook to find a curative agent for one of man's most horrible diseases. The first compound prepared and tested was labelled No. 1. It was found to have no value. The second, No. 2, after having been carefully prepared and tried out was no better. This process was repeated 605 times, each being a failure. The compounds often required weeks of careful, patient work to prepare, but the determination of this man was not to be hampered, and with the six hundred and sixth trial Ehrlich was able to give mankind the cure for the third great plague.

That man has become a master at killing his fellow-men was shown by the late war, but his works of destruction seem like child's play when compared with the destruction of bacteria. If one doubts it let him read the history of influenza, and he will find that it alone killed more human beings in two short years than man in four. Disease claimed four times as many lives during the Spanish-American War as did the enemies' bullets. During the nineteenth century man slew on the battlefield 19,000,000; yet, the little tuberculosis organism during the same time slew 34,000,000. Napoleon slew his tens of thousands, but the one discovery of Lister has saved more than this from the ravages of bacteria.

The individual interested in engineering achievements may have admired men's ingenuity in the fixing of atmospheric nitrogen by means of a gigantic arclight in a chimney through which is blown a current of hot air. The flame of this arclight has a diameter of seven feet and reaches a temperature of approximately 6300° F. The resulting product created by the flame when dissolved in water gives us nitric acid. In still another method, air is cooled to −194° C., the nitrogen boiled off, mixed with hydro-

gen in the proportion of one to three, heated to a temperature of 550° C., and this when passed under an extremely high pressure through a catalyzer electrically heated to about 550° C. gives as a result ammonia. And yet, how crude, and clumsy are these methods when compared with the engineering feats of the bacteria.

It is thus evident that bacteriology is a subject which is of immediate practical value to everyone and not merely a preparation for something that may be undertaken in the distant future. It is a subject that teems with interest and touches alike the every-day life of the one who returns to the shop, the office, the farm, or the home.

CHAPTER II

FOUNDING OF BACTERIOLOGY

Aeons ago bacteria gained a precarious foothold on bleak, primitive rock and manufactured acids which in time produced an abode fit for the growth of higher plants. Walcott claims to have found fossil bacteria in geological formations, the age of which is estimated to be 33,000,000 years. Even the sluggish, heavily armored dinosaurs were tormented and often fell prey to bacterial infections, as is shown by the fossils recovered from Permian deposits. From these fossils it appears that the same diseases tormented the early geological animals as attack the animals of today. However, we must remember that it is the diseases of the skeleton and not the vital organs which have left their records in the rocks. Be this as it may, we are certain that bacteria often ended the life of the ancient as well as the modern animal; and it was they who quickly removed in most cases the bodies of the dead plants and animals. Although bacteria were not known to man until the perfecting of the compound microscope in the last quarter of the seventeenth century, yet they manifested themselves centuries before. As early as the dawn of written history the putrefaction of meat, the fermentation of fruit juices, the decay of wood, and the sickening of animals were recorded, and numerous and interesting are the theories that were evolved to account for the observed facts.

Theories of Disease.—The ancient pagans accounted for disease in their theory of creation. Prometheus, to whom was entrusted the creation of man and the task of providing him and other animals with facilities necessary for their preservation was, as the legend runs, so liberal in providing for the lower animals that when man was reached nothing remained to bestow upon him. Man naked and empty-handed was left unable to compete with the lower animals; hence, man was given fire stolen from Jupiter with which to shape weapons. For this theft Pandora was sent as a punishment to Prometheus and his brother. In the house of these brothers was a jar in which were kept all

noxious articles that had been left over after the fitting of man for his new abode. Pandora in eager curiosity slipped off the cover, and out rushed the various plagues which afflict mankind.

The active imagination which caused the children of the race to fill the woods with fauns and satyrs, and the streams with nymphs and naiads, naturally caused them to see in the sick the actions of the demon. The Babylonians regarded disease as "the work of demons which swarmed in the earth, air, and water." With such a belief, it is not surprising to find the medicine men attempting to cure disease by two methods: (1) The enticing of the evil spirit from the body. This was attempted by the use of certain charms and incantations. (2) The removal of the demon by force. The methods resorted to in this connection varied but usually consisted of loading the patient with chains, whipping him, and giving him ill-smelling and ill-tasting drugs all of which were supposed to render the body of the victim an uninviting place for the evil spirit. Although this belief was confined primarily to the ancients, belief in it and its method of treatment persists among the savage and uneducated even at the present time.

A doctrine quite as old as the demonic theory is that all the joys and ills of man are determined by the position of the heavenly bodies, and even in modern times no less an authority than Noah Webster wrote a book in which he attempted to prove that epidemics are due to earthquakes and other terrestrial disturbances. Had he written after the great San Francisco Earthquake he could have used this as an example, for plague appeared after the earthquake. But what was the cause? In all probability the plague had existed in a sporadic form in San Francisco's "Chinatown" and in other places on the Pacific Coast, for many years. "Chinatown" was more or less isolated, but due to the earthquake the inhabitants of the locality were scattered through the city and with them also the plague-infected rats and fleas. Hence, there was a flare-up of the plague, which had it occurred during the Dark Ages would have spread like wild fire.

Hippocrates, the father of medicine, taught in the fifth century B. C. that disease is due to pestilential condition of the air. Gaseous substances were supposed to emanate from the soil, stagnant pools, marshes, decaying and putrescent substances, and crowded habitations. He vaguely realized that there was a relationship between water and disease for he adovcated the boiling of drinking water.

As a result of such teachings, aqueducts and sewerage systems were constructed by ancient Rome. The famous Cloaca Maxima, constructed 2700 years ago for sewage disposal, is in use today, and great aqueducts with their accompanying filters and settling tanks built at this early period are still to be found at the present time. That built by Quintus Marcius was fifty-six miles in length. However the people of later periods did not accept these theories of disease and during the dark ages they

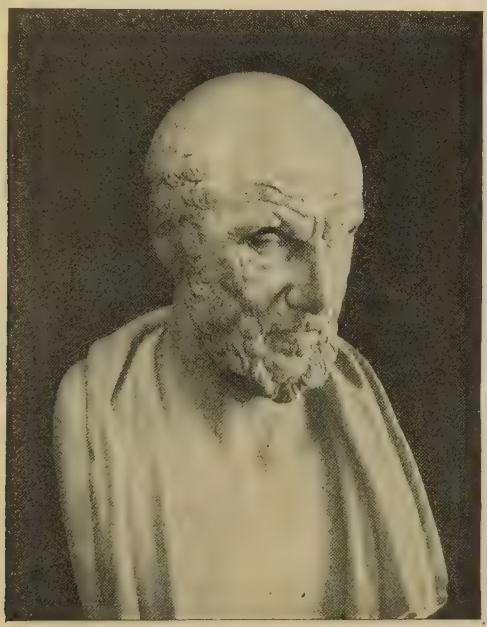


FIG. 1.—Hippocrates (460–370 B. C.). (*Greek marble bust in the British Museum.*)

were replaced by those of mysticism and the officials boiled witches instead of water to prevent disease. The result, obviously enough, was that sanitation gave way to filth and pestilence.

Discovery of Bacteria.—The germ theory of disease was probably evolved in the mind of an occasional genius many centuries ago, but it remained a theory until a comparatively recent date. It is possible that the Roman Catholic monk, Kircher, saw bacteria in 1671. He examined water, decaying matter, blood, and pus. In these he found what he called "invisible worms." He

even speculated concerning the relationship between these "invisible worms" and disease. Some bodies described were undoubtedly chains of blood corpuscles and the description of others is so inexact that it cannot definitely be stated that he saw bacteria.

Be this as it may, we know that bacteria were seen and accurately described soon after in 1683 by a Dutch linen draper, Anthony van Leeuwenhoek who is often called the father of bacteriology. During the course of his life he contributed to the British Royal Society one hundred and twelve papers dealing with various

FIG. 2.—Athanasius Kircher (1602–80).

FIG. 3.—Anthony van Leeuwenhoek (1632–1723).

Scientific topics. He also manufactured scores of microscopes, many of which were superior to any that had been made previously. With these he examined various things—raindrops, saliva, and putrefying substances. He found in every case living, moving animalcules which prior to his time had been unrecognized. We can imagine his joy and surprise from his statement: "I saw with wonder that my material contained many tiny animals which moved about in a most amusing fashion; the largest of these A (Fig. 4) showed the liveliest and most active motion, moving through the water or saliva as a fish of prey darts through the sea; they were found everywhere, although not

in large numbers. A second kind was similar to that marked B (Fig. 4) which sometimes spun around in a circle like a top. These were present in larger numbers and sometimes described a path that is shown in C to D (Fig. 4). A third kind could not be distinguished so clearly; now they appeared oblong, now quite round. They were so very small that they did not seem larger than the bodies marked E, and besides they moved so rapidly that they were continually running into one another. They looked like a swarm of gnats or flies dancing about together. I had the impression that I was looking at several thousand in a given part of water or saliva mixed with a particle from the teeth no larger than a grain of sand, even when only one part of the material was added to nine parts of water or saliva. Further, the greater part of the material consisted of an extraordinary number of rods, of widely different length but of the same diameter; some were curved, some straight as shown in F; they lay irregularly and were interlaced. Since I have previously seen animalcules of this same kind in water, I endeavored to observe whether there was life in them, but in none did I see the smallest movement that might be taken as a sign of life." Some of them he considered traveled with the speed of lightning and even "tore through each other."

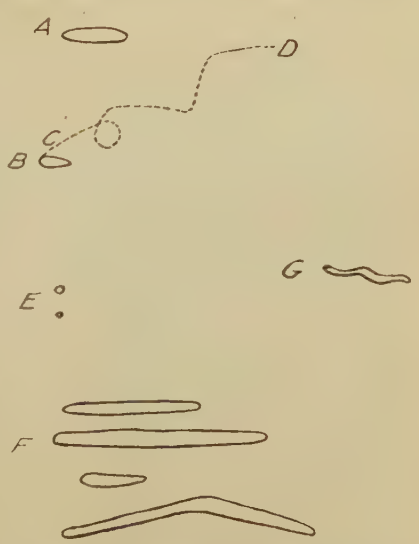


FIG. 4.—Drawings of bacteria. The dotted line C-D indicates movement of the bacteria. (*After Leeuwenhoek.*)

This patient worker, supplied with a microscope of his own make, gave a fairly accurate description of these minute forms of life. However, it did not awaken the world to even a faint realization of the marvelous invisible forms of life which were present in everything either as a blessing or as a curse. It did, however, revive a discussion which waxed long and furious as to whether life can spring spontaneously from dead matter or is always the offspring of preëxisting parents.

Spontaneous Generation.—Theories as to the origin of life are as old as the human race. The doctrine of spontaneous generation is one of them. The race is like a child in that during the early stages of development the imagination is the predominating instinct. We thus find the first descriptions of the origin of life highly imaginative. The Greeks looked on the Goddess Gea as the mother of mankind. In their glorious mythology they pictured men and women as springing into life from the stones cast on Gea. The Celts pictured the soil as peopled with gnomes and pixies, friends or enemies of mankind. Many ancient writers fancifully portrayed the transforming of dead into living matter. The Greek philosophers taught it. Aristotle wrote in 384 B. C.: "Animals sometimes arise in soil, in plants, or in other animals."

FIG. 5.—Paracelsus (1493–1541).

Three centuries later Ovid, in his dissertation on the Pythagorean philosophy, defends the doctrine of spontaneous generation, while Vergil (70–19 B. C.) in the Georgics, gives directions for the artificial production of bees.

Paracelsus (1493–1541), the Swiss medical philosopher who greatly confused fact and fancy, gives instruction for the making of homunculi. Certain substances are to be placed in a bottle; the bottle well stoppered and buried in a dung heap. Every day certain incantations must be pronounced over the bottle. In time, so Paracelsus declares, a small living human being (homunculus) will appear in the bottle. However, he naively admits that he never succeeded in keeping the homunculus alive after taking it from the bottle. Kircher even goes a step further and describes and pictures certain animals which he claims were spontaneously produced before his eyes through the influence of water on fragments of plants. Cardan (1501–1576) thought that water gave rise to fish and animals and that it was the cause of fermentation.

An Italian, Bononani, tells of a wonderful transformation which he claims to have witnessed. Rotten timber which he

rescued from the sea produced worms; these gave rise to butterflies; and strangest of all, the butterflies became birds and flew away. Gradually these grotesque fanciful opinions concerning spontaneous generation were abandoned, and it was believed that only the lower plants and animals, seaweeds, algae, lichens, lice, mites, and maggots, could develop spontaneously. Even today we can find fairly intelligent individuals who believe that mites and lice can develop without parents and that the hair from the tail or mane of a horse will change into a worm or snake if placed in water and exposed to light and warmth.

FIG. 6.—Francesco Redi (1626–1694).

Everyone took it as a self-evident fact that maggots originated spontaneously from decomposing meat or cheese until an Italian poet and physician, Redi (1626–1694), took the simple precaution of screening the mouths of large jars containing meat so that flies could not enter. The flies attracted by the odor deposited their eggs on the gauze, and it was from these eggs that the so called "worms" arose.

By the middle of the sixteenth century the theory of the spontaneous generation of mice, scorpions, and maggots had been proven untenable. But the problem was far from being settled as regarded other forms of life, for when Leeuwenhoek with the aid of his lenses discovered the various living and moving animalcules in raindrops, saliva, and many putrefying substances, it was at once believed that the riddle of life had finally been solved. Now anyone provided with the microscope, could easily demonstrate for himself the spontaneous generation of microscopic eels in vinegar, or produce myriads of different and interesting living creatures in simple infusions of hay or other organic material.

Origin of Bacteria.—Following the discovery of bacteria, theories became rife as to their origin. Two champions of the theory of spontaneous generation who now took up the controversy were Needham and Buffon. Needham, a Catholic priest, evolved the theory that a force called "productive" or "vegeta-

tive" existed which was responsible for the formation of organized beings; while the great naturalist, Buffon, elaborated the theory that there were certain unchangeable parts common to all living creatures. After death, according to his conception, these ultimate constituents were set free and became very active, until with one another and still other particles they gave rise to swarms of microscopic creatures.

Needham experimented somewhat and in 1745 took decaying organic matter and enclosed it in a vessel. This he placed upon hot ashes to destroy any existing animalcules. On examining the contents of the flasks he found microörganisms which had not been there in the beginning. Later in 1769 Spallanzani repeated the same work, for he felt that Needham had not exercised sufficient care and that the organisms got in from the outside. Spallanzani boiled the material for one hour and kept it in hermetically sealed flasks. He wrote: "I used hermetically sealed vessels. I kept them for one hour in boiling water, and after opening and examining their contents, after a reasonable interval I found not the slightest trace of animalculæ, though I had examined the infusion from nineteen different vessels."

But the believers in the theory of spontaneous generation were not convinced. They claimed that boiling altered the character of the infusion in such a manner that it was unable to produce life. Voltaire, with his characteristic satire, took up the fight at this point and ridiculed the operations of the English clergy "who had engendered the eels in the gravy of boiled mutton," and he very wittily remarked: "It is strange that men should deny a creator and yet attribute to themselves the power of creating eels." But this was a controversy to be settled not by ridicule but by experimental evidence. Spallanzani answered his opponents by cracking one of the flasks so that air could enter. Decay soon set in. This, however, was not sufficient to overthrow the popular belief, for the claim was made that in the sealing of the flasks air was excluded, and that was essential to the generation of these forms of life. This objection was taken up by a number of very ingenious investigators. Schulze, in 1836, passed air through strong acids and then into boiled infusions and failed to find any living organisms in the infusion, whereas Schwann passed the air through highly heated tubes with the same results. These methods were criticized by their opponents who claimed that the chemical alteration of the air subjected to such drastic treatment had been responsible for the

absence of bacteria in the infusion. The work of Schroeder and Dusch (1853) was more convincing, for they found that it was sufficient to simply stopper the bottles with cotton plugs; the air passed in, but the microörganisms were held back by the cotton and the contents of the flasks kept free from contamination.

Even this was not sufficient to overthrow the belief of spontaneous generation, for as late as 1859 Pauchet revived it in a book in which he heaped experiment upon experiment and argument upon argument spiced with logic and sarcasm in favor of spontaneous generation. In this he was opposed by Pasteur who collected the floating particles of air surrounding his laboratory in the Rue d'Ulm and subjected them to microscopic examination. Pasteur sowed these particles in sterilized infusion and obtained abundant crops of microscopic organisms. He repeated and confirmed the experiments of Schwann which had been contested by Pauchet. He showed that the cause which communicated life to the infusions was not uniformly diffused, that in the workshops and crowded streets of Paris living organisms were numerous, whereas on the tops of high mountains and glaciers the air was usually free from life. He showed that beef tea sterilized in flasks with the neck bent like that of a swan did not spoil even though exposed to the atmosphere. As late as 1922 there was exhibited in the United States one of these flasks of beef tea which it was claimed Pasteur had prepared over fifty years before; it was still clear and free from life.

Nearly all Paris was at his famous lecture at the Sarbonne April 7, 1864, in which he portrayed vividly and with a touch of scorn for his adversaries his conclusions concerning the origin of bacteria. He said: "There is no condition known today in which you can affirm that microscopic beings come into the world without germs, without parents like themselves. They who allege it have been the sport of illusions, of ill-made experiments, vitiated by errors which they have not been able to perceive, and have not known how to avoid." In a passage of singular beauty he described himself watching and imploring his flasks to give him a sign of life, but they would not—"for I have kept from them, and am still keeping from them, that one thing which is above the power of man to make; I have kept from them the germs which float in the air; I have kept from them life."

In England the controversy was waged between Bastian and Tyndall. The latter proved that in an atmosphere devoid of

dust, as on the tops of mountains and in some ingeniously constructed boxes used by him, perishable substances such as beef tea, if sterile when placed in such an atmosphere will keep for an indefinite period. However, every now and then he found that the contents of a flask would spoil, even after it had been carefully stoppered and boiled. This remained a stumbling block in the way of those who maintained that life sprang only from life until the year 1865 when Pasteur demonstrated that many bacteria may pass into a resting stage in which they can withstand conditions that quickly kill them when in the vegetative form. Eleven years later Cohn of Breslau investigated very carefully organisms in this resting or spore stage, and today we know forms of microörganisms that are able to withstand boiling water for sixteen hours without being killed, and others even resistant enough to endure for many hours a 10 per cent solution of carbolic acid.

Thus was established the principle that life springs only from life, from the viewpoint of the welfare of the human race, the most momentous discovery ever made by man, for on it are reared those three sciences which have done so much to prevent, alleviate, and cure human ills: bacteriology, pathology, and surgery.

Early Classifications of Bacteria.—Having discovered new objects, it is human nature to ask: From whence did they come? What are they? How shall we classify them? We have seen that it required two hundred years to answer the first question and during all this time and even at the present time there are efforts being made to answer the others. Leeuwenhoek thought that the microscopic forms of life which he had discovered were animals, and inasmuch as they grew in various infusions, he called them "infusion-animalcules." Although he gave no specific names and his description is often faulty due to his crude microscope, it has been stated that he distinguished twenty-seven kinds or "species" of his infusoria.

Linnæus (1707–1778), the great Swedish botanist, in his classification of plants, with what appears to be a little outburst of temper, assigns these minute organisms to a division of his great class "vermes" and gave to them the name of "chaos." O. F. Müller (1730–1784), the Danish naturalist, was the first to attempt to bring order out of this "chaos." He figured and named about a hundred more organisms than had been described by Leeuwenhoek. He used the Linnæan binomial nomen-

clature. The genus Vibrio comprised that strange mixture of species which we today know as the bacteria.

Fifty years after the appearance of Müller's work, Ehrenberg published a magnificant volume on the "infusion-animalcules." In this he had 1,500 exquisite drawings of various organisms This really marks the first serious attempt at classification, and in it appeared numerous names which have been retained even to the present time. Here for the first time appeared the name bacterium, and from it has originated the name bacteriology, the name of the science dealing with these microscopic forms of plant life.

There appeared almost simultaneously the work of Schroeder and Cohn. The work of the former dealt with the pigment-producing bacteria and their growth on potatoes, thus giving to the subject a new cultural medium. Cohn divided the microscopic organism into two classes: the one comprising those multiplying by budding and the second those multiplying by fission. To the organisms belonging to the second class he gave the name bacteria and placed them in the plant kingdom. He further classified bacteria into four tribes, and there appear in this classification generic names which are used even today in much the same manner as used by Cohn.

Since the time of Cohn many changes have been made in the classification. New ones have been proposed, many with meritorious parts but all with defects; and today, even though scientists have come nearer to the coveted goal, no classification has yet been devised that has proven satisfactory enough to be universally accepted.

Development of Methods.—The advancements in bacteriology have been intimately associated with the development of the microscope. The writings of Pliny, Plutarch, and Seneca make reference to simple magnifers which probably were used in making the fine engravings found on ancient gems. During the early years of the fourteenth century lenses were used by ingenious Italians to improve failing sight, but the discovery of the principle underlying the compound microscope was made by Johann Janssen and his son, Zacharias, during the latter part of the sixteenth century. But the manufacture of the first compound microscope is usually attributed to Robert Hooke in 1665. This microscope was crude and did not give a clear image; hence, Leeuwenhoek used simple lenses which he himself ground into proper shape and curvature and mounted between two perforated pieces of silver. Greater magnification, however, was desirable,

and various efforts were made to improve these crude instruments. It was due primarily to the efforts of Joseph Jackson Lister that the compound microscope became a practical working instrument. He, with the aid of the celebrated optician Tulley, devised means for the combination of lenses of crown glass with others of flint glass so that the refractive errors of one were corrected by the other. With such an instrument it was possible to get a highly magnified image free from distortion and fringes of color that had heretofore been such a drawback in the use of the compound microscope. Since then many improvements have been made in the instrument, and it has become a vital factor in the advancement of bacteriology.

FIG. 7.—Carl Weigert (1845–1904).

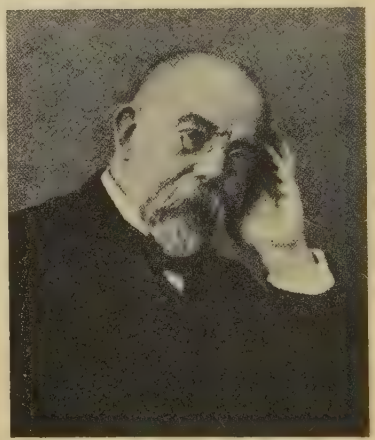


FIG. 8.—Robert Koch (1843–1910). (*Courtesy of Captain Henry J. Nichols, U. S. Army.*)

We have found the two great landmarks in the history of bacteriology to be the dscovery of bacteria by Leeuwenhoek and the recognition by Pasteur that putrefaction, fermentation, and decay are due to microörganisms which have parents as do other plants and animals. A third great advancement was made in 1876 when Weigert used some of the analin dyes to stain the body of bacteria, thus making it possible to see and study the shape and structure of organisms which may occur in body tissue, soils, milk, and water. Still a fourth milestone on the path

of bacteriological research was passed when the immortal Robert Koch devised the gelatin-plate method for obtaining pure cultures of bacteria. He added to liquefied portions of sterile gelatin small quantities of the material containing the bacteria which he wished to study, shook the mixture to distribute the organisms uniformly throughout the liquid gelatin, and spread it on a covered sterile plate. The bacteria were fixed in isolated spots where they multiplied until they had formed colonies large enough to become visible to the naked eye. These colonies, each the offspring of a single cell, could furnish only one kind of bacteria. These could be fished out with a sterile platinum needle, studied under the microscope, grown in different media, perchance in soil, or the body of susceptible animals until their shape, function, and various other characteristics were understood.

Many writers take 1882 the year in which solid media were first used, as the birth of the new science bacteriology. The reason for this becomes clear when we review the methods used up to this time in obtaining pure cultures of microörganisms.

Pasteur, in his wonderful observations on yeast, obtained his pure cultures of yeast by feeding these organisms on a food especially suited to their needs. In this they grew rapidly and in time crowded out those less suited to the medium. This was a long and tedious process and could be successfully used only where organisms had great physiological differences.

Klebs (1873) tried to obtain pure cultures by a fractional method. He simply removed those organisms which grew most luxuriantly to another flask, making special cultures of these and so on until comparatively pure cultures were obtained. Lister (1878) and Nägeli (1879) obtained pure cultures by diluting and transferring to special flasks decreasing amounts of the medium. Theoretically, if the dilution is carried far enough some flasks will receive only one organism from which there will develop a pure culture. But it can readily be seen that such methods depend on chance, and chance is against the worker where two organisms develop in close association with each other.

Prior to this, Roberts (1874) and Cohn (1876) had obtained pure cultures of some spore-bearing bacteria by subjecting the medium to a temperature of 100° C. at which all the non-spore-bearing forms were killed. This method was very limited in application; hence, it was not until the introduction of the solid cultural method by Koch (referred to above) that a scientific method of studying bacteria became available.

CHAPTER III

THE CONQUEST OF DISEASE

Man has lived on this planet for thousands of years, yet until the middle of the eighteenth century he had not subdued a single disease. Since that time he has worked marvels. Many diseases have been controlled—some completely conquered. Portions of the earth's surface which had been stripped of its animal life and made barren by disease have been restocked and made a fit place for the production of human food. Regions which had been seething hotbeds of pestilence have been converted into the most healthful spots on earth. Since the birth of bacteriology this world has been made a more decent home for man to live in, love in, marry in, rear his children in, and to die in at a riper age; for during this time man's natural span of life has been doubled.

Smallpox.—If humanity of the past could march in military array before the eyes of humanity of today arranged in armies according to the cause of their demise, what a spectacle it would be! One of the greatest armies would be composed of the victims of smallpox. Foremost among its ranks would be the countless billions of prehistoric cave men, followed closely by the millions from India. There would be divisions on divisions of Chinese from the pre-Christian era to the nineteenth century. During the short period of twenty-five years no less than fifteen million Russians joined the ranks; yes, probably every seventh child born in Russia for centuries would be found in this army. Sixty million men, women, and children joined it during the eighteenth century, whereas during the same period only nineteen million joined the army who lost their lives on the battlefield. Since the dawn of history millions from Europe, Asia, and Africa have joined each century. At the beginning of the sixteenth century we should find millions from the new world marching hand in hand with those of the old world. In the ranks we should see the savage and the civilized, the ignorant and the educated, the strong and the weak, the old and the young, the prince and the pauper, the king and the queen, and as we review the procession we should indeed conclude that no one

was safe from the ravages of smallpox. We need but turn over a few pages in the annals of history to understand the terrible devastation, havoc, and dread that this disease has caused among mankind since the beginning of time. "It granted no favor to old, it smote the middle-aged, it struck down the young, it scarred the unborn babe. None were so lowly as to be passed by without notice, none so powerful as to enjoy immunity. Not only did it stalk through the narrow alleys, but it walked abroad on the boulevard. It lay on the toiler's cot of straw and parted the purple curtains of the emperor's bedstead. Long before socialism was heard of, smallpox proclaimed 'special privileges to none.'" It is no wonder then that "in the course of ages, the human race accumulated such fear of the eruptive fever that when the epidemic swooped upon the crowded communities of civilization there were times when sick infants cried in vain for mothers who had fled. When its pimpled visage appeared in the untamed forest an Indian father would call his family together, speak to them of the evil spirit which was torturing the tribe, and pointing to the dehumanized features of those already attacked would exhort his children to escape a similar fate by falling upon their own daggers, promising them if they lacked the courage that he himself as a last proof of his devotion would do the deed of mercy and at once follow them to the happy land." Truly, in those days, no mother could count her children until they had had smallpox for "from smallpox and love few remained free."

Continuing to view our visionary army we note at the close of the nineteenth century a marked difference in the number and kind of individuals who march in the files and ranks of the smallpox army. It no longer contains one-tenth of the human race, but at times only a few stragglers. It is no longer made up of all sorts and conditions of peoples, but there appears to have been a selecting action. The doctors and nurses who so often helped to swell the ranks in the previous centuries have disappeared, and the ranks are made up more of the ignorant and less progressive. Some nationalities, as the English, Irish, Scotch, German, Swedish, Philippine, and Porto Rican, have nearly disappeared from the ranks, whereas the Belgian, Russian, Austrian, Spanish, and American races are there in greater numbers. Why this change in number and in nationality?

This question carries us back to the last quarter of the eighteenth century, when in the office of a country doctor a young girl made the chance remark, "I cannot take that disease (small-

pox) for I have had cowpox." These words found fertile soil in the mind of young Jenner who, as he went about his work, pondered over the statement and wondered if there could be any truth in the strange belief of this country girl. He not only pondered over the subject, but he sought out and listened to the gossip of the cowherders and milkmaids as if he were collecting the folklore of a disappearing race. He learned to tell the true form of cowpox from the spurious types. He gleaned all the information on the subject that it was possible for him to get. He even persisted in talking of it at the meetings of a medical society to which he belonged until the members became bored and threatened his expulsion if he persisted. After twenty-five years of study and meditation the opportunity presented itself for a test of this perplexing problem. A dairymaid, Sarah Nelmes, had been pricked by a thorn and while milking a cow had become infected with cowpox. On May 14, 1796 Jenner took matter from her hand and placed it in a small scratch on the arm of an eight-year-old boy, James Phipps. Six weeks later the boy was inoculated with smallpox, a method that was universally used at that time to protect against it. The boy showed no ill effect from the inoculation. This test was repeated in various places by different individuals, always with the same results. Jenner published his findings in a small 75-page book. In it is an engraving of the hand of Sarah Nelmes—"the hand that helped to halt a plague."

FIG. 9.—Edward Jenner (1749–1823). (*From the painting by Sir Thomas Lawrence.*)

After considerable opposition from the haters of progress, vaccination spread over the world. Civilized and uncivilized, the great and the lowly, availed themselves of the great protective factor. Jenner lived to see that disease of which it has been said, "In those stricken days, if a messenger had come from heaven and standing on earth's highest hill had clarioned to all mortals: 'From the long role of evils I shall remove one disease; which shall it be?,' one universal voice would have ascended in answer,

the desire of kings blending with the prayer of peasants, the cultured accents of the scholar mingling with the cry of the man of the streets: 'Smallpox.'" In those nations in which vaccination has been made compulsory smallpox has disappeared. In the other nations it is still prevalent, and as the inhabitants forget the horrors of the past they neglect or oppose vaccination until reminded by the appearance of the grim destroyer. No one who has read the history of the past and the present has the temerity to claim that vaccination does not protect against smallpox. But some do say that there is danger in vaccination. To those, it can be said without fear of contradiction that the one who submits to vaccination is not endangering life nor limb and that the fear of vaccination is uncalled for.

The change in the number and personnel of the smallpox victims has come about through the battle of a country doctor against the king of diseases with "a little virus on the point of an ivory lance." So great was his victory that if every one were vaccinated and revaccinated, as medical experience shows to be necessary, the recruits to the smallpox army would absolutely cease, and in a few generations the horrible scourge (smallpox) would be a historical curiosity.

Pebrine.—Pebrine, a disease of the silkworm and named from black pepper-like spots appearing in the body of the infected silkworms, was one of the first diseases successfully controlled by Pasteur. He commenced work in 1865 "when the silk industry had been going from bad to worse. France, Italy, Spain, Greece, Turkey, China were all involved; the loss and the misery, down in the south of France, were terrible: it needed only a few more turns of the screw to squeeze the life out of this great national industry." It was undertaken by Pasteur as a patriotic duty at the request of an old friend, Dumas, one who admired, had confidence in, and could forsee the greatness of Pasteur. For five years Pasteur struggled on without sufficient equipment, facing criticism and discouragement, suffering the loss of a kind father, of two loving children, and he himself brought near the brink of the grave by a cerebral hemorrhage which left him a cripple. In spite of these obstacles, in the end Pasteur won. He found that the black spots in the body of the diseased silkworms were disease-producing microörganisms. After long and careful study he proved that the disease is not only contagious but hereditary. He found that diseased moths lay diseased eggs from which developed diseased silkworms.

These soon died or were useless in the production of silk. The germs left on leaves or in dust soon perished, but the ones in the eggs survived. Pasteur proved that the disease might be ended by the destruction of all diseased eggs. To this end he devised the process of placing each female moth when ready to lay its eggs on a separate piece of linen. After it had laid its eggs and adhered to the linen it was dried, removed from the linen, ground up in water, and the water examined microscopically. "If 'corpuscles' are found in it, the whole of the eggs of this moth and the linen on which they were laid are burnt; if no 'corpuscles' are found, the eggs are kept to be hatched in due time and yield healthy silkworms." When the method was announced the cry came back: "It is impractical." To this Pasteur impatiently answered "Don't tell me that anyone wants anything simpler than a preventive method which is just to put your eye to the eyepiece of a microscope after pounding a moth in a mortar with a few drops of water—mere child's play, taking an hour or two to learn." But the prejudice, falsehood, and bickering were overcome only by Pasteur personally taking over an old badly infected, valueless (insofar as the production of silk was concerned) plantation and transforming it into a profitable, productive disease-free establishment. By so doing he demonstrated the practibility of his method, rescued the silk industry, and saved for France something like four millions of dollars yearly.

FIG. 10.—Louis Pasteur (1822-1895).

Anthrax.—The second disease to be conquered was anthrax, a disease highly fatal and widely prevalent among the lower animals. With man it is quite rare and less fatal. It is referred to in both sacred and profane history, modern and ancient. No one knows when or where it had its origin, Until the last quarter of the eighteenth century man stood in helpless astonishment before its fatal and rapid action. Its causative organism was the first pathogen to be seen by man, the first to be obtained in pure culture, and one of the first to be conquered. The

organism was seen and described by Pollender in 1855, but he failed to prove that it was the cause of anthrax. Devaine, in 1863, all but reached the coveted goal which was attained by the young German bacteriologist, Robert Koch, in 1876. Koch obtained the organism in pure culture and proved it to be the specific cause of splenic fever (anthrax).

In 1877 Pasteur set out to discover a cure or a prevention for the disease. For years he struggled on. He traveled through infected districts, listened to the farmers' stories, studied its natural history, and discovered the organism causing it in the body of earthworms which had fed on the carcasses of animals dying from anthrax (thus, showing how it may be carried about in the soil). He grew the microörganism in test tubes, and proved that it could be transferred from flask to flask and still produce the disease in susceptible animals. He subjected it to abnormal temperatures, injected it into the bodies of animals and found that they did not contract the disease. Such cultures rendered the animals immune, that is, the disease could not be caused in animals subjected to this treatment. After repeated verification of the results, and after most careful analysis, he announced to the world that he had found a prevention for anthrax. His announcement fell on the ears of scoffers and skeptics. One veterinary journal in 1881 carried the following: "Will you have some microbe? There is some everywhere. Microbiolatry is the fashion; it reigns undisputed; it is a doctrine which must not even be discussed, especially when its Pontiff, the learned M. Pasteur, has pronounced the sacramental words, 'I have spoken.'" Ridicule, sarcasm, and even personal abuse was not to deter the man who had fought through sorrow and illness and who knew that he had discovered a great truth. Great, indeed, was the astonishment and surprise when Pasteur announced that he would submit the question to a decisive public test. Fifty sheep were to be given the virulent anthrax bacteria. One-half of them were to have been previously protected by the vaccine, while the other half were not. Pasteur predicted that the twenty-five unprotected sheep would perish, while the protected ones would survive. On May 5 the animals to be protected received their first dose of the vaccine. Twelve days later they were given a stronger dose. On May 31 both groups were given the virulent anthrax bacteria. On the afternoon of June 2 the crowd, composed of farmers, veterinarians, physicians, journalists, and scientists from near and far, met to learn whether

Pasteur was a dreamer or a prophet. What they saw was one of the most spectacular scenes in the history of peaceful science, a scene which Pasteur afterwards declared "amazed the assembly." Scattered about the enclosure, dead or dying, were the unprotected sheep. Twenty-two were dead; the other three survived only a short time. The vaccinated sheep were in perfect health!

The results created a tremendous excitement throughout France. It was not only a victory for Pasteur, but it also meant a great saving to his noble countrymen, and it placed the name of his beloved war-crushed France one notch higher in the hall of fame. To mankind it marked a victory of far-reaching importance in the conflict which man is waging against disease.

Rabies.—In the beginning rabies, sometimes called hydrophobia, was confined to the lower animals. As man domesticated animals he shared their diseases. It is therefore, likely that even during the childhood of the race the cry of the mad dog struck terror to the heart of man. This great fear of the disease has come from two causes: first, the mortality which is practically 100 per cent; and second, there are few, if any other afflictions in which the suffering is so intense. So great is this that no sane man would witness it a second time were he not needed to aid the unfortunate victim. Pasteur sensed these facts, and oh, how he longed to bring relief! He was now past the prime of life, an invalid, and had written his name securely in the history of science. Many a less ambitious man would have rested on his laurels and enjoyed, as the world sees enjoyment, his declining years. Not so with the maker of a new science, for in December 1880 he entered upon the arduous self-imposed task of love: to find a preventive, or a cure for the awful malady. Not only was the work difficult, but it was beset with grave personal danger. Pasteur did not fear the danger, and he hoped to overcome the difficulties. The causative organism was unknown. All that was known was that the disease was conveyed by the bite of a rabid animal.

Pasteur firmly believed it to be caused by a microörganism. He had helped to overthrow the idea of spontaneous generation and prove that every microörganism is the offspring of a similar one; hence he reasoned the virus causing this disease should be found in the mouth of the afflicted animal. The saliva of the diseased child and dog was inoculated into susceptible animals. The results were various. At times the animal

died of rabies, but more often it died of septicemia (blood poison). The blood was tried with no better results. Pasteur reasoned: "Inasmuch as rabies is a disease of the nervous system, the cause should be sought in the nerves." Parts of the spinal cord of animals dead of the disease were grafted under the skin of the rabbit. The results were indefinite. They were placed on the brain of experimental animals and rabies invariably resulted, but the latent period (the time between the introduction of the material and the appearance of the symptoms) varied. How could the latent period be made a constant? The spinal cord of one rabbit dead of the disease was used on a second, and this on a third, and so on. The latent period grew shorter and shorter, until in time the rabbits always died between the sixth and seventh days. Pasteur had obtained what he called "fixed virus" in distinction to the ordinary or "street virus."

After numerous and varied experiments, he found that the spinal cord of an animal dead of the fixed virus could be dried under appropriate conditions and used to immunize animals against rabies. The treatment consisted of giving the animal as soon after being bitten as practicable a salt solution of the dried cord and later on another solution of cord which had been dried for a shorter period. In time, the experimental animal could receive the undried disease cord without ill effects. The animal had become immune. In principle, it is as if an individual were to receive two months hence a fatal dose of morphine. He is given today and each day thereafter small but increasing doses of the drug. When the date for receiving the fatal dose is reached, he takes it with impunity.

The treatment was tried on thousands of animals which had been bitten at varying times, under different conditions, and also on animals which had been given the nervous tissue of rabid animals. The results were alway the same. When given just before or soon after the infection the animal never developed rabies. Still Pasteur hesitated to try it on man until circumstances crowded it upon him. The occasion soon offered itself.

On Monday morning, July 6, 1885, an Alsatian woman brought her child to Pasteur's laboratory. The boy had been attacked while on his way to school by a mad dog. He had been knocked down and bitten in fourteen places. When rescued by a bricklayer his wounds were found to be deep and covered with the saliva of the rabid animal. He was taken to the village doctor who carbolized his wounds and sent him to Pasteur who examined

the boy and was greatly touched by his suffering and prospects, for wounds such as he had received invariably proved fatal. Pasteur hesitated to give him the treatment. The mother insisted. Pasteur's advisers intimated that the boy's death would be upon Pasteur if he refused to treat him, and the mother absolved him from all responsibility if the treatment were given. Against the advice of friends Pasteur began the treatment upon the boy, shortening the time between the treatments in an effort to secure a cumulative effect. The child in perfect confidence ate well, slept soundly at night, and played about the room during the day. Pasteur spent sleepless nights and anxious days watching for the least ill symptom which might appear. The crisis passed. Pasteur had conquered hydrophobia! In October he saved his second patient, the young shepherd Jupille, who to protect some small boys had collared and killed a mad dog. In so doing he was frightfully bitten. Six days later he received the treatment and was saved.

The news of these two cases rapidly spread and brought a rush of patients to Pasteur. Soon Pasteur institutes appeared in available centers throughout the world, and today the mortality of the malady which formerly was nearly 100 per cent has been reduced to less than one per cent.

Pasteur Honored.—Pasteur had been the subject of criticism and distrust. His discoveries had been received with skepticism and forebodings of evil. He had struggled on at times almost alone, amidst poverty, sickness, and sorrow with the sincere desire to help his beloved France and especially to alleviate the ills of man. He lived to realize many of his hopes and to be the most honored man of his age. He was the recipient of honors from learned societies. Scholars paused to pay him tribute. Even the laity recognized his greatness, as was shown by the great popular vote that was taken in which Pasteur was acclaimed the greatest man of his age winning over such heroes as Napoleon and Victor Hugo. When subscriptions were called for the erection of Pasteur institutes in which his work could be continued they flowed from all quarters of the globe—the offerings of the rich and the poor, the great and the lowly. On his seventieth birthday delegates from scientific societies and public bodies met in France to do him honor. The President of the Republic escorted Pasteur to the rostrum where he was lauded as one of the greatest heroes and benefactors in human history. Three years later when he died his remains were laid away amidst

the tributes and sorrows of a grateful world in a magnificant chapel. In the vault over his grave stand erected four great white angels—Faith, Hope, Charity, and Science.

Yellow Fever.—For centuries some of the most fertile areas of the New World were uninhabitable to the white race, or if perchance any of this race settled in those regions the appearance of yellow fever forcefully reminded them that they must move or die. Ninety times it invaded the United States, often enacting scenes which were the counterpart of the plague days in London. This is seen from the following description by Carey of the 1793 yellow-fever epidemic in Philadelphia:

"The consternation of the people of Philadelphia at this period was great beyond all bounds. Dismay and affright were visible in the countenance of almost every person. Of those who remained, many shut themselves in their house and were afraid to walk the streets . . . The corpses of the most respectable citizens, even those who did not die of the epidemic, were carried to the grave in the shafts of a chaise, the horse driven by a negro, unattended by friends or relatives, and without any sort of ceremony. People hastily shifted their course at the sight of a hearse coming toward them. Many never walked on the footpath but went into the middle of the street to avoid being infected in passing by houses where in people had died. Acquaintances and friends avoided each other in the streets and only signified their regard by a cold nod. The old custom of shaking hands fell into such disuse that many shrank back with affright at even the offer of the hand. A person with crepe, or any appearance of morning, was shunned like a viper, and many valued themselves highly on the skill and address with which they got the windward of every person they met. Indeed it is not probable that London, at the last stages of the plague, exhibited stronger marks of terror than were to be seen in Philadelphia from the twenty-fourth or twenty-fifth of August till pretty late in September. While affairs were in this deplorable stage and the people at the lowest ebb of despair, we cannot be astonished at the frightful scenes that were enacted, which seem to indicate a total dissolution of the bonds of society in the nearest and clearest connections. Who, without horror, can reflect on a husband deserting his wife, united to him perhaps for twenty years, in the last agony—a wife unfeelingly abandoning her husband on his deathbed or parents forsaking their only children without remorse—children ungratefully fleeing from their parents and

resigning them to chance without an inquiry after their health—masters hurrying off their faithful servants to Bush-Hill even on suspicion of the disease, and that at a time when, like Tartarus, it was open to every visitant but never returned any—servants abandoning the tender and humane masters who only wanted a little care to restore them to health and usefulness—who, I say, can even now think of these things without horror?"

For four centuries the narrow Isthmus of Panama was regarded as the white man's grave. It is claimed that the first railroad constructed across it cost a human life for each tie. Ferdinand de Lesseps, who undertook to construct a canal across the Isthmus, was forced to abandon it, not through lack of engineering skill, but because he did not recognize the part played by the mosquito. His men died like flies. Common laborers, nurses, doctors, and engineers alike fell prey. One vessel brought over from France eighteen young engineers. Within a month after their arrival all but one had died. Before the work was finally abandoned thousands had lost their lives, and it was hard to keep enough well to care for the sick. Eighteen per cent of all the men employed had died, and many more were helpless. Twenty years later a canal was constructed and that with a mortality of slightly less than sixteen per thousand! Today the mortality is less in the Canal Zone than in many of our large cities. The change is due to the discovery of the cause and method for control of the yellow fever.

Even as early as 1797 Dr. Rush of Philadelphia noticed excessive numbers of mosquitoes during yellow-fever epidemics. In 1848 Dr. Nott of Mobile, Alabama, suggested that yellow fever was disseminated by some insect "that remains very close to the ground." Five years later a French physician attempted to prevent the disease by disinfecting the bite. In 1881 Dr. Carlos Finlay formulated the mosquito theory of the transmission of yellow fever. During the rest of the century the hopes of man rose and fell with every theory that was promulgated or whenever a suspected organism was isolated only to be shown in the end that it was not the cause of the disease.

When General Wood was made military governor of Cuba in 1898 he immediately cleaned up the city Santiago and the surrounding provinces. He succeeded in making the city an exceptionally clean place, but his efforts were futile in so far as the eradication of yellow fever was concerned. Conditions became so bad that in 1900 General Nelson O. Miles had a commission

appointed "for the purpose of pursuing scientific investigations with reference to the infectious diseases prevalent on the island of Cuba." The commission was composed of the bacteriologist, Walter Reed, and Doctors James Carroll, Jesse W. Lazear, and Aristide Agramonte.

They built two mosquito-proof houses. In one of these soldiers lived for a period of twenty-one days. By night they slept in bedding soiled with the vomit and excreta of sick and dying yellow-fever patients. This was repeated with three different detachments. Not one developed this or any other disease!

FIG. 11.—Walter Reed (1851-1902).

The second house contained two rooms separated by a screened partition. All of the furnishings which went with the house had been disinfected and were perfectly clean. Volunteers were placed in the two rooms. In the one mosquitoes were liberated which had fed on the blood of yellow-fever patients. All mosquitoes were excluded from the other: Fifty per cent of the men in the mosquito ward developed yellow fever and not one in the other ward, although they were separated only by a screen. On August 27, 1900 Doctor Carroll permitted an infected mosquito to bite him. His account of the experiment is as follows: "The insect, which had been hatched and reared in the laboratory, had been caused to feed upon four cases of yellow fever, two of them severe and two mild. The first patient, a severe case, was bitten twelve days; the second, third, and fourth patients had been bitten six, four, and two days previously, and their attacks were in character mild, severe, and mild, respectively. In writing to Dr. Reed on the night after the instance I remarked jokingly that if there were anything in the mosquito theory I should have a good dose; and so it happened after having slight premonitory symptoms for two days I was taken sick on August

31 and on September 1 I was carried to the yellow fever camp. My life was in the balance for three days." Dr. Lazear had previously submitted to an experimental bite without effect; later he had allowed a mosquito, which settled on his hand while he was taking blood from a yellow-fever patient, to take its fill. After a short incubation period he developed the disease from which he soon died. Dr. Agramonte writes: "How can I describe the agony of suspense which racked our souls during those six days? It seemed to us as though a life was being offered in sacrifice for the thousands which it was to contribute in saving. Across the span of thirteen years the memory of the last moments come to me most vividly and thrilling, when the light of reason left his brain and shut out of his mind the torturing thought of the loving wife and daughter far away and of the unborn child who was to find itself fatherless on coming into the world. Tuesday, the twenty-fifth of September, saw the end of a life full of promise; one more name, that of Jesse W. Lazear, was graven upon the portals of immortality."

After further tests the following principles were established: Yellow fever is not contagious, nor is it conveyed by means of fomites. It is conveyed only by a species of mosquitoes (Aëdes egypti) which, in order to be dangerous, must feed on the blood of a yellow-fever patient during the first three days of his illness, after which the insect is harmless to man for a period of twelve days. After this period its bite will convey the disease to the non-immune person. Hence, to prevent the disease in man it is necessary to prevent the infection of the mosquitoes by man and to eradicate them by destroying their breeding places.

Soon after this discovery the danger of yellow fever in the United States was virtually eliminated and probably the first great plague to entirely disappear will be yellow fever.

Other Plagues Conquered.—The discovery of antitoxin for diphtheria has reduced the mortality of this disease from 30 to less than 3 per cent, and the introduction of the toxin and antitoxin, foreshadows its absolute control. Typhoid fever is all but controlled. Asiatic cholera and plague are fast being swept from the earth. Typhus fever and a number of other diseases are within the control of man. All this has come within three generations! What will the future bring?

Future Work.—One may think from the preceding that in this field of science there is little to be done, but this is not the case for there are diseases still unconquered. "Measles bends over

every cradle; the bacillus that Koch saw in 1881 is as much a menace today as it was twenty-five centuries ago when Hippocrates called consumption the most dangerous disease; the swift and sudden onslaught of pneumonia carries desolation in its wide trail." Cancer is on the increase. Man stands helpless before the onrush of influenza. Pneumonia, and meningitis demand their yearly toll. Other diseases claim their tens of thousands each year, while the physicians stand helplessly by and watch the losing fight of their patients. Surely, some future Pasteur will bring relief!

CHAPTER IV

MICROÖRGANISMS BECOME THE ALLIES OF MAN

Bacteria at first were looked upon as curios which probably represented the origin of life; later, as the enemies of man; and still later it was learned that they are not only useful to man but absolutely essential to his existence. A few have become outlaws and prey upon life, but the overwhelming majority administer to the needs of life.

Fermentation.—Since the dawn of history man has been interested in the wonderful process known as fermentation, and although many an ingenious theory has been formulated to explain it little more than theory existed until the classic work of Pasteur on fermentation appeared about 1837. Pasteur claimed that all forms of fermentation were due to the action of microscopic organized cells. An idea such as this, even at this late date, did not go unchallenged, for we find no less illustrious workers than Helmholtz and Liebig opposing it. The latter even scoffed at such an idea, writing: "Those who pretend to explain the putrefaction of animal substance by the presence of microörganisms reason very much like a child who would explain the rapidity of the Rhine by attributing it to the violent motions imparted to it in the direction of Burgen by the numerous wheels of the mills of Venice."

Pasteur's early work was in chemistry. "He prepared a double salt of racemic acid, a sodium ammonium recemate, and let it crystallize. These crystals were of two forms, one having the facet of the commercial acid and the other having the facet proper to the unknown acid. He separated the two forms of crystals, picking them out one by one. Solutions were prepared from each and tested with a polariscope. They were different. The one form rotated the plane of the polarized light to the right, the other turned the plane, through an equal extent, to the left. He had separated the crystals by hand; later he allowed microörganisms to feed upon a solution of the two forms. One was used by the organism as food; the other was left untouched. And so Pasteur passed from the field of chemistry to

the realm of bacteriology. Due to this discovery there developed explanations of molecular structure and the use of microörganisms for the separation and identification of closely related compounds.

Pasteur devoted much of his time for twenty years to a study of fermentation. During this time he obtained in pure cultures many microörganisms and proved that each produced its own peculiar results or products of life activity. He showed that diseases of wine were due to foreign organisms and that heating to a comparatively low temperature made it possible to keep indefinitely products with their desirable flavors. Thus originated the modern process of pasteurization. His carefully planned and executed experiments which together with the logical conclusions drawn from them, put the brewing industries on a sound scientific basis, in place of the old cut-and-try method of the preceding periods. These discoveries alone saved France $20,000,000 yearly; and what is more important, they proved that without microörganisms there would be no fermentation, no putrefaction, no decay of any kind except by the slow process of oxidation!

This was only one of the many processes studied by Pasteur. In 1857 he gave to the Lille Scientific Society his paper on lactic-acid fermentation. Four years later he discovered another fermentation which occurred after lactic fermentation and known as butyric-acid fermentation. He noted the relationship between this and the rancidity of butter, and also demonstrated that the ferments grow only in the absence of air, thus establishing the fact that there may be life even in the absence of free oxygen. To these microörganisms he later gave the name of anaërobes. In 1867 Pasteur taught the vinegar makers of Orleans how to speed up the fermentation of vinegar. The next year he taught France how to keep her rough wines from going sour. In 1871 he took a huge east-end brewery in London by surprise and showed them the impurities of their yeast and the evil effect it was having on the liquor. When driven from his laboratory to the home of his childhood during the siege of Paris he spent his time studying the fermentations in the tannery. "He would ask endless questions, trying to discover the scientific reason of every process and every routine. Whilst his sister was making bread he would study the raising crust, the influence of air in the kneading of the dough, and, his imagination rising as usual from a minor point to the greatest problems, he began to seek for the means of

increasing the nutritive powers of bread, and consequently of lowering its price."

The work of the Danish investigator Hansen in obtaining pure cultures of yeast is significant. He succeeded in seeding cultures with a single cell and separating one species from another. He proposed methods for the differentiation of species, worked out their life cycle, and proposed a classification which in recent years has been universally accepted.

Fermentation in Bread-making.—We do not know when fermentation first entered the baking industry. Probably before the dawn of modern history man discovered that crushed grains mixed with water and kept in a warm place underwent a desirable change which today is known as fermentation. Leavened bread was used in the days of the ancient Israelites, for we are told how in the flight of the Israelites out of Egypt "the people took their dough before it was leavened, their kneading troughs being bound up in their clothes." The art of leavening passed from the Egyptians to the Greeks, and from the Greeks to the Romans whose conquests and colonies extended the art. At first it consisted in a normal fermentation of the dough allowing it to become sour, but to hasten the process it became the custom to add to new dough a portion of old fermented paste or "leaven." Later yeast was substituted for the piece of leaven, and the process of bread-making passed from the realm of chance to that of order. But before it became a science much was yet to be done. Various questions arose, many of which are still unanswered today. Of the numerous strains of yeast, which are best for bread-making? Is it possible to breed still better strains? What is the best food on which to grow them so they will produce ideal results when mixed with the dough? How can we accelerate their growth and retard the growth of the natural-occurring organisms of the dough? Bacteria are concerned in the making of "salt-rising" bread. What are the species concerned? Who will prepare and place on the market pure cultures of these that possibly will equal or even surpass the yeast in producing a bread of fine flavor and ideal texture? Surely the future will answer these questions.

Butter and Cheese.—Some races of the human family, especially the Chinese and Japanese, prefer sweet-cream butter, whereas the majority prefer sour-cream butter. In order to obtain the latter product, the cream is allowed to sour due to the production of lactic acid within it produced by bacteria. Until

recently, and in many cases even today, the practice has been to allow the cream to sour spontaneously. Due to the great variation of the natural microflora, this process results in products varying in flavor and often being defective. But when in 1890 Weighmann introduced into the dairy industry the process of artificially souring cream by the aid of pure cultures of selected races of lactic-acid bacteria the process became controllable, and today the dairyman can produce day after day butter of the same flavor. This same process within recent years is being applied to the making of cheese, and in the future the kind of cheese produced by the dairyman will be governed by his knowledge and the cultures he uses and not by the location of the factory, as in the past.

Other Fermentation Processes.—Certain essential changes in the extraction of indigo blue from the indigo plant are now known to be due to a bacillus which naturally occurs on the leaves of the plant. Much of the valued flavor of tea, cocoa, and tobacco is developed within them due to a fermentation process. The tanning of hides, the retting of flax, and the changes in the silo are all due to microörganisms. Each year man is learning more and more how to extend and control these processes.

Soil Bacteriology.—It was a well-accepted maxim with the older writers of agriculture that "corruption is the mother of vegetation." One of the most illustrious of their number, Liebig (1855), taught that plant and animal tissues decayed in the soil with the formation of ammonia which he considered highly essential to plant growth. Part of this ammonia according to his belief was transformed into nitric acid which also served as plant-food.

Long before the nature of the process by which plant residues and manures are transformed into nitrates was understood the method was used to supply the large quantities of gunpowder consumed in the almost incessant wars of Europe. In the eighteenth century the artificial production of saltpetre in beds of decaying organic matter reached a high degree of perfection. Especially was this true in Sweden, Switzerland, and France where nitrates were collected as a part of each farmer's tax. In the year 1777 the French Government issued special instructions for its manufacture in which there was stressed the form of pit to use, the covering of organic matter, the arrangement for the free entry of air, the optimum amount of moisture, and the necessity of a base to neutralize the acid as formed.

Boussingault, a French chemist, in the years between 1860 and 1878 became interested in the natural-occurring "nitre beds," especially those of Peru and Ecuador. He did work which established the origin of the nitrate as coming from the organic matter of the soil. It was considered, however, that the transformation was a chemical change produced through the interaction of ozone or active oxygen on the ammonia coming from the organic matter. The soil was supposed to act as a catalyzer, thus accelerating the speed of the reaction.

During the sixties and seventies great advances had been made in bacteriology. It had been definitely established that bacteria bring about decay, putrefaction, and fermentation, and it was conceivable that they were the active agents in changing the ammonia within the soil. Pasteur had expressed the opinion that nitrification, the changing of ammonia into nitrates, was a bacterial process, but nothing definite was known on the subject until 1877, when Schlösing and Müntz were surprised by the peculiarity in action of sand filters used in purifying sewage.

A continuous stream of sewage was allowed to trickle down a column of sand and limestone at a rate such that it required eight days to pass. The first twenty-one days the ammonia in the sewage was unaffected. Then it began to appear as nitrates, and in time only nitrates issued from the filter. If it were a chemical process, nitrates should be found at once. Why must twenty-one days elapse before the ammonia was transferred into nitrates? In attemping to answer this, they found that the process could be stopped by the addition of chloroform and started again by adding the extract of fresh soil. Nitrification was thus shown, to use their expression, to be "due to organized ferments."

The succeeding thirteen years represent the history of a race among the leading scientists of Europe and America to determine to whom should go the honor of first obtaining and studying in pure culture the microörganisms which possessed this important property of transforming ammonia into nitrates.

Warington of England proved that no matter what compounds of nitrogen are supplied to a plant as manure they are rapidly transformed within the soil into nitrates. Furthermore, he found that ammonia is first changed to nitrite and then into nitrate.

However, up to the time Winogradsky took up the subject, all attempts to isolate such organisms had failed. After long trying

experiments which demonstrate the keen, untiring ingenious nature of this investigator, he gave to the world nitrifying organisms. He found they would not grow in the ordinary media that had been used by previous workers; the media must be free from organic matter.

It has been known for generations that uncropped soil increases in fertility and that legumes differ from other plants in their nutritive requirements. Less ancient, however, is the knowledge that uncropped soil may gain in nitrogen or that legumes with the aid of bacteria may get their nitrogen from the soil.

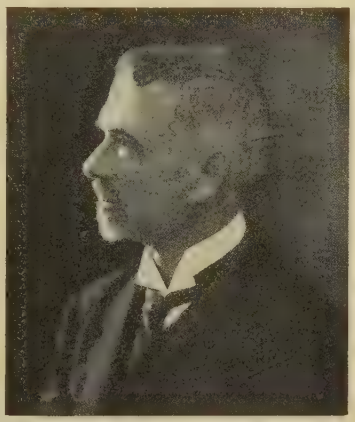


FIG. 12.—S. Winogradsky. (*From Waksman's, Principles of Soil Microbiology. Williams and Wilkins.*)

In the middle of the nineteenth century Boussingault wrote: "Vegetable earth contains living organisms—germs—the vitality of which is suspended by drying and re-established under favorable conditions as to moisture and temperature." He also hinted at the fact that these microörganisms take nitrogen from the air and change it so that it can be used by the growing plant. He spread out thinly 120 gm. of soil in a shallow glass dish and for three months moistened it daily with water free from nitrogen compounds. At the end of this time he found that it had gained nitrogen but had lost carbon. Thirty years later Hellriegel and Wilfarth solved the apparently hopeless problem of the nitrogen nutrition of leguminous plants. They found that bacteria live in little nodules on the roots of legumes and manufacture nitrogen compounds which they give to the higher plants in exchange for carbohydrates and other nutrients needed by bacteria. Since then we have learned much concerning the relationship of plants to free and combined nitrogen of the air and the soil.

In 1901 Beijerinck discovered large yeast-like microörganisms which live free in the soil and take from the atmosphere nitrogen and build it up into complex compounds within their bodies. Every year since this date the number of microörganisms which have been found to possess this property has been added to, until

today many microörganisms possessing this important property are known.

Terrific battles are being waged in the soil between the microscopic plants and the microscopic animals. This was the conclusion reached by Russell and Hutchinson in 1909. Since that date each year has added new knowledge concerning organisms already known or else added new ones to the list of beneficial organisms, until we now know that the soil is teeming with organ-

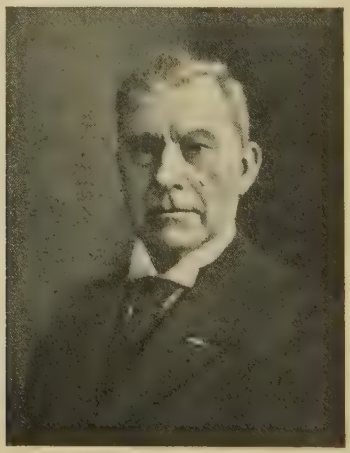


Fig. 13.—M. W. Beijerinck. (*From Waksman's, Principles of Soil Microbiology. Williams and Wilkins.*)

isms most of which are beneficial. Some of them, however, are without significance, and a few of them are injurious. Microörganisms not only deal with nitrogen and organic matter of the soil but they act on sulfur forming sulfuric acid; they form acids which liberate phosphorus, potassium, calcium, and the many other foods needed by the plants. It is the problem of the worker in soil bacteriology to learn how to speed up the work of the beneficial and to suppress or weed out the injurious. Advances have been made in this realm, but for the most part it is a virgin field which awaits the coming of the untiring worker who cares to thrust in his sickle and reap. For, truly, the harvest will be rich enough to satisfy the most fastidious.

CHAPTER V

BACTERIA: OCCURRENCE AND FUNCTION

Bacteria rule the world. Man is dependent upon them from the day of his birth until the hour of his death, and even when that hour comes they are present. They turn out the light and take possession of the body. They are man's most useful servants and his most destructive master. This being true, one is prone to ask: What are bacteria? Where do they occur? What are their functions?

What are Bacteria?—Bacteria are very minute single-celled living beings devoid of roots, leaves, and stems. They are so small that they can be seen only with the aid of a powerful microscope; consequently, they are often spoken of as micro-örganisms. This term includes not only bacteria but all forms of life so small as to require the microscope in their study. They are often referred to in common usage as germs or microbes. The early investigators considered them animals; hence, they referred to them as "animalcules." The terms bacteria, germs, or microbes usually suggest to the layman a minute animal, and we often hear them spoken of as "bugs" even today. On examination we find that bacteria have many of the characteristics of animals. Some have the power of independent motion. All are devoid of green coloring matter, chlorophyll; hence, most of them are compelled to live upon complex foods as do the animals. Their general structure, their methods of growth, their formation of threads and spores, and their similarity in general to some of the lower forms of plant life, such as the green algae, have caused the biologist to class them as plants. However, it is impossible to make a clear-cut distinction between some microscopic plants and some microscopic animals. This is not surprising, for long before these simple forms of life were known the terms, plants and animals, were invented to distinguish familiar living objects, such as elephants and trees, insects and mosses. Haeckel has suggested that to obviate this difficulty we consider bacteria neither as plants nor as animals, but that we create a new kingdom to be known as the Protista, into which

we could place all of the lower forms of life. However, this does not solve the problem, for we would then have to draw two lines of demarcation in place of one. The important thing to remember is that bacteria are at the very foot of the ladder, the simplest forms of life, and hence, partake of the characteristics of both plants and animals. For this reason and for convenience, scientists have agreed to consider the bacteria with the plants.

The plant kingdom consists of four great divisions: (1) the seed plants or Spermatophytes, (2) fern plants or Pteridophytes, (3) mosses or Bryophytes, and (4) thallus plants or Thallophytes. The Thallophytes have neither roots, stems, nor leaves. There are two main divisions of the Thallophytes: (A) the algae which possess the green pigment chlorophyll and are, therefore, able to manufacture their own food from the carbon dioxide of the air; and (B) the fungi which lack chlorophyll and, consequently, are obliged to obtain their food from living plants or animals or from dead organic materials. To this division belongs the great array of bacteria, molds, yeasts, blights, rusts, toadstools, mushrooms, and similar plants.

Nor is it an easy task to differentiate nicely between bacteria, yeasts, and molds. They may roughly be distinguished from each other as follows: Bacteria are single-celled plants containing nuclear material which is intimately diffused throughout the cell. They multiply by transverse fission—a pinching in two at the center of the organism. Sometimes they are united into long chains which are easily separated into their separate links. Each link is a complete organism. Yeasts are usually larger than bacteria. They are single-celled organisms and have a definitely organized nucleus. This sets them off from bacteria. They multiply by budding, that is by the formation of buds, or small protuberances, which appear on the side of organisms, enlarge, and two daughter cells are formed. Molds consist of numerous cells. These are a mass of interwoven threads, each thread being composed of a number of cells which function as a unit.

Where Do Bacteria Occur?—Bacteria are widely distributed, occurring nearly everywhere. They are found in all soils, the number varying with the kind of soil, quantity of plant and animal debris present, moisture, and treatment. They decrease in number with depth, but many soils of Western America contain numerous microörganisms even at a depth of ten feet. Although they occur in air, it is not their natural home, as under

ordinary conditions they cannot grow and multiply in it. The number and variety found in air vary with a number of factors location, moisture, dust, movement of the atmosphere, and the presence or absence of injurious gases. The atmosphere of some high mountains or glaciers and the air over the ocean far from shore is paractically free from bacteria. City and country air also differ from each other in the number and kind of bacteria which they contain. There is a great variation in the air of buildings. They are especially numerous where dust is plentiful.

Most natural waters contain great numbers of bacteria. In sewage and polluted water they are especially numerous. They occur only in small numbers or not at all in deep wells and springs, shallow wells, ponds, and streams draining inhabited districts often contain millions in one cubic centimeter. They are even found in salt lakes and the ocean. In these waters they function even at great depths. The kind and number vary with the composition of water and the original contamination. Great Salt Lake with its large quantities of common salt contains few organisms, whereas the Great Lakes in the central part of the United States contain many. A turbid stream, like the Mississippi which contains the drainage from many cities, has a great variety and number of bacteria in opposition to the clear, rapid, flowing water of uninhabited mountainous regions.

Milk, as secreted by the cow, is practically free from bacteria, but as it is drawn from the udder it always contains them. The number and kind vary with specific cows. Some healthy cows may give milk with as few as twenty bacteria in a cubic centimeter, whereas the milk in the case of others may contain thousands, while that of diseased cows may contain millions. During the milking process milk receives germs from the coat of the cow, the clothing of the milker, the buckets, strainers, and other things with which the milk comes in contact. Often by the time it reaches the consumer it contains millions of bacteria in every cubic centimeter.

Bacteria are found on the surface but not on the inside of undamaged fruit and vegetables. The normal tissues, sap, and heartwood of plants, are free from them. If injected into them the bacteria may survive for some time, but the tissues must die before they can serve as food for bacteria. All food except that just cooked contains bacteria, the number and kind varying with the nature and age of the food.

Living as we do in a world filled with bacteria, we can expect to find them on the surface of the skin and mucous membranes. Usually they live there, feeding on the dead matter of the surfaces. At times they work down into the pores of the skin and manifest themselves as blackheads, or as the more painful affliction boils. Normally the infant enters the world free from bacteria, but bacteria soon settle on his skin. They penetrate the nose and mouth; the first respiratory movements and cries carry them into the respiratory passages; and between the tenth and seventeenth hour they have reached the intestines. From the minute of birth until the time of death there is a constant struggle between the individual and the microbes, and one individual has jokingly remarked, "Microbes get us at last." This is strictly true, for most individuals die of bacterial diseases long before the allotted three score and ten years have elapsed, and the bodies of even those who die from accident or old age, unless cremated, are decomposed by bacteria.

Ordinarily, the deeper respiratory passages contain few bacteria, but it has been proven that at times even the tuberculosis bacilli can penetrate with the inspired air to the bottom of the lungs.

On account of its acidity, yeasts and molds flourish better in the stomach than do bacteria. However, at least thirty different kinds of bacteria have been described as occurring in the stomach. Many of these have attracted special attention on account of the belief that their presence may favor other more injurious species.

The intestines, on account of their alkaline reaction and the partly digested condition of their contents, are a great reservoir of bacterial activity. In the upper part there are few, but by the time the descending colon is reached billions of bacteria are present. Sometimes they constitute one-third of the total dry contents of the intestine. The health of the individual is determined by the number and kind of bacteria, and at least one writer has suggested that our very personality is governed by the microbes we carry about with us.

The normal tissues and the blood of animals are usually free from bacteria. Microörganisms are rarely found on certain healthy mucous membranes, such as those of the kidneys, bladder, and lungs. Occasionally they pass through the skin or mucous membranes of the digestive tract after which they may be found for a short time in the blood. This is especially true during the

height of digestion, and we find at this time large numbers of white corpuscles swarming into the intestinal mucosa ready to pick up and devour any bacteria which may enter. In this manner the body is protected against invaders. In certain diseased conditions the blood and tissues of man and lower animals become filled with bacteria. Just before and soon after death they rapidly invade and tear down the body.

Functions of Bacteria.—We are living in a world teeming with bacteria. They cover the surface of the body and swarm in the alimentary canal. Hence, we are prone to ask: Do those in the alimentary canal help us, or would we be better off without them? Early experimenters found that chickens, frogs, and turtles if hatched free from bacteria and fed on sterile food did not grow normally, whereas, other experimenters have found that the guinea pig could be reared to maturity on sterile food with its alimentary canal free from bacteria. Still more recent investigators claim to have found the digestive tract of the white bear, seals, reindeer, eider ducks, and penguins in the Arctic regions to be entirely sterile; hence, bacteria are not essential to these animals. They may assist cows, sheep, and horses in their digestive processes, but it is doubtful if they are essential in the alimentary canal of man. Their real significance comes in the fact that we are living in a world filled with them. They cannot be kept out of the alimentary tract. The normal flora does no harm but does produce products which are inimical to the growth of many injurious ones. As a consequence of the knowledge of this fact, considerable attention has been given to the favoring of the beneficial bacteria in man. The good effect which sometimes follows the use of sour milk and buttermilk is due to their carrying into the intestines beneficial bacteria together with food favorable to their growth. Sometimes individuals are given cultures of *Lactobacillus bulgaricus*, or still better, *Lactobacillus acidophilus*. Little is to be gained by this procedure unless at the same time the diet of the individual is made to favor these species.

The great Russian bacteriologist, Metchnikoff, claimed that the rate with which man ages is determined not by the years he has lived, but by the bacteria which inhabit his digestive system; and Morris, in his interesting book "Microbes and Men," even argues that they control the personality of man. They are the governors of his whims, fancies, likes, and dislikes. Just as alcohol partaken of by man usually causes first elation, later

depression, and finally helplessness, likewise bacteria at first invigorate and later depress. All are familiar with the statement: "I never felt so well in my life as I did just before I came down with this sickness; in fact, I felt unusually well and spoke to my friends about it." Parents are continually telling the doctor: "My boy was never so full of life as the day before he came down." Dr. Morris further argues that the optimist, the pessimist and the neurotic are what they are, melancholic and morose because of the bacteria which they carry about. A rabbit given a certain small dose of strychnine will show no ill effects if left quietly by itself, but if such a rabbit be disturbed it goes into convulsions. Likewise, the neurotic made so by the absorption of bacterial poisons, if left quietly by himself is docile and contented, but if slightly irritated become morose, sullen, cross or even pugnacious, depending upon circumstances.

Bacteria play numerous wonderful parts in the many changes going on in this world. This is true to such an extent that it is hard to conceive of our living in a world without bacteria. Examining the soil from which man draws—either directly or indirectly—his clothing, food, and other necessities of life, we find it to be a veritable garden of minute plants which have been at work within it long before man began to till the soil. What a wonderful change they produce in transforming the bleak, barren rock into a fertile field from which man can gather his delicious berries, or beautiful flowers; his golden sheaves of wheat, or his deep green nutritious loads of hay!

Changes in temperature cause small cracks to appear even in the more resistant rocks. Bacteria being of microscopic size soon penetrate these small crevices, and produce there carbonic, nitric, and other acids which gradually dissolve away the rock. The microörganisms attack even the most minute fragments reducing them continually to smaller and smaller particles. If one picks from a fertile soil a speck of rock, it will be found coated with a fine film of these minute plants. They have drawn on it for their food, and at the same time they have rendered it soluble so that the wheat, alfalfa, or corn plant can feed upon it. Bacteria continue their work long after the rocks have been changed to soil. Each day they liberate a little more plant-food for the growth of the crop. During the year they are able in a fertile soil to liberate enough plant-food for the production of a good crop. When manure is applied to a soil it not only supplies food for the growing plant, but it also carries to the soil beneficial

bacteria and supplies those already there with food. These in turn liberate a little more iron, sulfur, potassium, or phosphorus, and the farmer reaps from his soil a bigger and better crop.

One of the essential elements for plant production, and the one which is usually in the soil in the smallest quantities, is nitrogen. This, unless it be applied to the soil in the form of the expensive fertilizer known as nitrates, must be prepared for the plant by bacteria. The microörganisms that carry on this particular form of work are the true kitchen maids of the soil, and the farmer finds his crops measured directly by the speed with which they prepare the meal. If they are active and prepare the right food, other things being favorable, there will be a good crop; but if they fail in their work, though everything else may be ideal, yet there can be no crop.

An examination of soils has shown that those which are most fertile contain the greatest number of beneficial bacteria. If the soil is rich in plant residues and has the right quantity of water and sufficient heat many bacteria will be found there changing the plant residues into gases and acids which act upon insoluble substances and render them soluble. One group of bacteria changes the plant and animal proteins into ammonia, and their activity can often be detected by the odor of ammonia coming from stables or manure piles.

However, most plants cannot use nitrogen in the form of ammonia; it must be changed into nitrates. This is done by two species of bacteria. One group feeds upon ammonia and manufactures nitrous acid. Nitrites are poison to plants; hence, if these were left unchanged at this stage we would have no plants; but it so happens that another class of bacteria feeds upon the nitrites as fast as they are formed and yield for us nitric acid. This reacts with the various minerals of the soil. This form of nitrogen is now ready to be taken up by the growing plant and manufactured into nourishing food, beautiful flowers, delicious fruit, or fragrant perfumes for the human family.

So far only the plant-food already in the soil and the changes through which it passes have been considered. However, the farmer is concerned with the substances his soil lacks and which must be added in order to get good crops. In many cases the lacking element is nitrogen. One notes from fertilizer quotations that this element costs fifteen cents a pound or over if purchased in the form of sodium nitrate, ammonium sulfate, or dried blood. On making a simple calculation we find that at this price it

would cost fifteen dollars for enough of these substances to produce 100 bushels of corn; eleven dollars for enough to produce fifty bushels of wheat; and seven dollars and fifty cents for enough to produce one ton of alfalfa hay.

In these calculations it has been assumed that one recovers in the form of corn, wheat, or alfalfa every pound of the commercial nitrogen which has been applied, which as a matter of fact is an utter impossibility; hence, we have to look elsewhere to obtain nitrogen for our growing crop. Here also bacteria come to the rescue.

There are seventy-five million pounds of atmospheric nitrogen resting upon every acre of land. However, none of the higher plants have the power of taking this directly out of the air. One family of plants, the Leguminosae, which includes peas, beans, alfalfa, clover, and many others, if properly infested by bacteria, has the power of using this atmospheric nitrogen. Under this condition and with these plants nitrogen no longer remains the limiting element of crop production, for the bacteria which live within small nodules upon the alfalfa are master chemists. Within their tiny laboratory they can bring about changes which man can imitate only imperfectly with costly machinery and powerful electric currents. In some of the experiments carried on at the Illinois Experiment Station these minute organisms were found to increase the value of the first cutting of alfalfa hay $27.80 an acre. That is, considering the nitrogen in the alfalfa at the price we would have to pay on the market for an equivalent quantity of nitrogen in the form of a commercial fertilizer! If these crops were plowed under, the fertility of the soil would be increased to just that extent. One writer has said of them: "They not only work for nothing and board themselves, but they pay for the privilege." This is strictly true, for all they require is a plant on which to grow and a well aerated moist soil containing limestone as well as the essential plant-food other than nitrogen. They cannot work in an acid soil nor can they produce something from nothing.

There is another class of nitrogen-gathering organisms within the soil which differs from the above in that it lives free in the soil and gathers nitrogen. Under ideal conditions these organisms may gather quite appreciable quantities.

It is quite possible that much of the benefit derived from the summer fallowing of land is due to the growth within the soil of this class of organisms which store up nitrogen for future

generations of plants. It has been discovered that they are more active and found in greater numbers in soil treated in this manner. All of the work which the farmer puts upon the soil in rendering it more porous reacts beneficially upon these bacteria, because they not only love atmospheric nitrogen and oxygen, but because they must have them.

Bacteria are the universal scavangers that lick up the dead bodies of plants and animals as completely and nearly as rapidly as fire. Were it not for these organisms the world in time would be filled with never-changing organic matter. The plant residues, trees, and animal bodies would remain in the soil, and with them the carbon which is required by the green plant. Bacteria, in getting their required energy, are continually liberating carbon, thereby permitting this important element to start again on its constructive journey. If carbon and nitrogen could but speak, what wonderful tales they would tell! The chemist, the bacteriologist, and the farmer each would be wiser, for many of the changes through which carbon and nitrogen pass due either to the action of the one-celled plants, bacteria, or the higher plants, wheat and alfalfa, are so complex that the scientist even with his apparently magical methods cannot follow them.

Bacteria are the architects which have built and are building many of the chalk deposits of the world. Certain bacteria which live in the warm portions of the sea effect the reduction of nitrates in the water to ammonia. The ammonia, combines with carbon dioxide, produced by other bacteria. This reacts with calcium sulfate in the sea water with the formation of calcium carbonate or chalk. This product separates out in the form of small spherical or elongated grains, and accumulates in deposits known as oölite. Such deposits are forming today in great abundance off the coast of Florida. Probably in early geological ages this method of chalk formation was very important.

The plumber at times meets hard masses of rust-like deposits which partly or completely fill the water mains. To him these are a curiosity or a nuisance, but to the bacteriologist they recall the method by which some of the iron deposits were formed. In the past some of the warm waters covering the surface of the earth carried large quantities of iron salts. Iron-bacteria grew in these waters and extracted from the water the iron. This they deposited in a coating that surrounded their bodies and called a sheath. When they died the iron was deposited; thus, we have the origin of at least part of the bog iron-ore of today.

Bacteria are found by the thousands in every drop of water in the cesspool where they lie in wait and quickly devour the material which enters. Paper, bone, and wood are quickly changed into gases and ash. The repulsive poisonous substances soon disappear under their magic wand, and it is only a short time until the water again becomes pure.

Bacteria grow in cream, making churning easier. They give to butter its characteristic flavor. They cause the ripening of cheese; thus, giving to it the peculiar flavor in opposition to the insipid taste of the fresh cheese.

Some writers even argue that the disease-producing microbes are beneficial, for these slay plants and animals; consequently the materials of which their bodies are composed may be used by others. It is, however, more logical to consider that they formerly were harmless, or that they were beneficial, as they grew on the surface of plants and animals, and decomposed dead material. It can well be imagined that they later reached and were carried about on the bodies of animals and still later started to invade the body. In the struggle between them and their host some were routed and disappeared, while others became true parasites preying upon plants and animals. These are now the outlaws in bacterial society. Such bacteria produce poisons which are injurious to man or lower animals, but man is learning to turn their poisons against themselves in the production of vaccines and serums which are used to render man and the lower animals immune to their attacks.

These are only a few of the many ways in which bacteria benefit the human race. They are at work in the silo, rendering the food more palatable and nutritious for cattle. They give to sauerkraut and pickles their flavor. They take part in the tanning of skins, the retting of flax, the curing of tobacco, the flavoring of coffee and cocoa, and the making of indigo from the indigo plant. In short, they help us in a thousand and one ways which we little suspect. Even today only a few of the changes have been fully studied; hence, one of the most instructive and interesting tasks set for man is to learn what these processes are and how to govern them.

CHAPTER VI

MORPHOLOGY OF MICROORGANISMS

Everyone recognizes that there are different races of mankind. Even the untrained naked eye perceives different varieties of plants and animals. The lynx-eyed lens has revealed to the scientist bacteria differing in form and structure. The branch of bacteriology which deals with the form and structure of microörganisms is known as morphology.

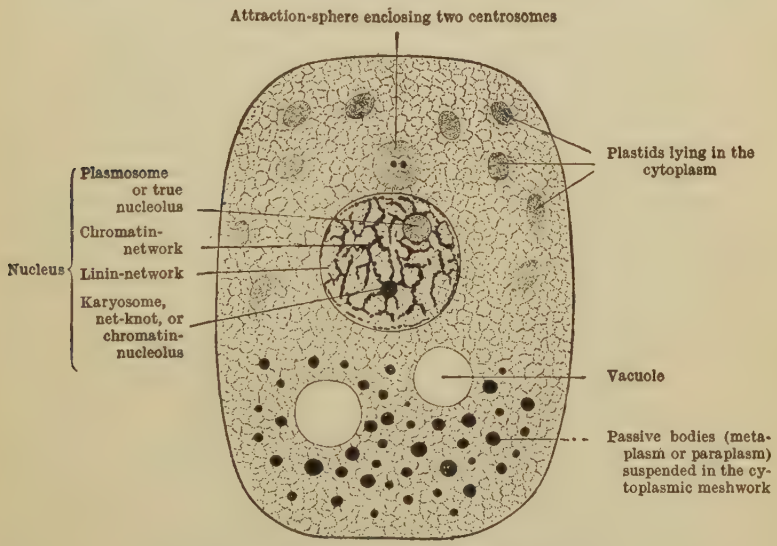


FIG. 14.—Diagram of a cell. (*Wilson.*)

The Cell.—One can divide an animal into different parts as head, trunk, arms, and legs. These in turn can be further divided into eyes, ears, nose, and so on. Now, if one examines with a microscope any of these parts he finds that they in turn are composed of still smaller parts called cells. Therefore, plants or animals, man or the microbe are all composed of a cell or cells. These cells vary greatly in shape. Some are cylindrical; others

are oval; still others are globular. In size they vary from the tiny bacterium which requires a great magnification before it can be seen, to the muscle or nerve cell which is measured in inches or even feet. If one were to examine more minutely a cell it would be found to be surrounded by an outer retaining membrane—the cell wall. Just beneath the cell wall in the majority of cells is a supporting structure of less dense material—the ectoplasm. Still deeper within the cell is a clearer less refractive part containing besides granules of food and waste many cell organs. This is known as the endoplasm. Within the center of the cell is a still more dense portion which stains with the analin dyes more deeply than the rest of the cell. This is the nucleus. The cell then, is the unit of life and may be defined as a mass of protoplasm containing a nucleus. However, when one includes the bacterial cell he finds it necessary to modify this definition so as to read "a cell is a limited mass of protoplasm containing nuclear material." The reason for this modification is that bacterial cells probably have their nuclear material diffused throughout the body of the cell; and as a consequence, the definition for bacteria must make clear that they are unicellular microörganisms devoid of a definite organized nucleus.

Shape of Bacteria.—Bacteria are single-celled microörganisms possessing the very simplest conceivable structure. Although there are thousands of different bacteria having great variations

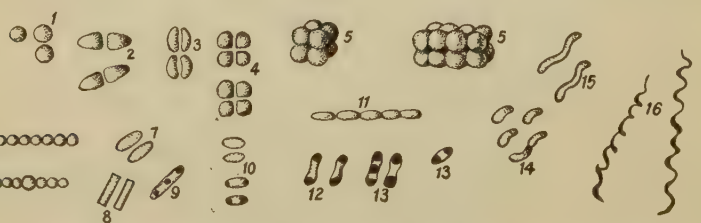


FIG. 15.—The normal types of bacteria 1–6, cocci; 7–13 bacilli; 14–16, spirilla; 1, micrococcus; 2 and 3, diplococci; 4, tetracoccus; 5 sarcina; 6, streptococcus (the lower chain includes an arthrospore); 7 and 8, bacilli; 9, 10, 12, 13, bacilli with various granules; 11, strepto-bacillus; 14, vibrio; 15, spirillum; 16, spirocheta treponema. (*From Kendall; Bacteriology. Published by Lea and Febiger.*)

in properties, yet they can all be grouped as cylindrical, globular, or spiral. The cylindrical or rod-shaped bacteria may be likened unto an unsharpened lead pencil. Some have rounded ends, others straight, while still others have the ends hollowed out. The

size also varies, some being so short that it is impossible to tell even with the best microscope whether they are cylindrical or

FIG. 16.—Large bacilli. (*After Harrison.*)

globular organisms. All rod-shaped bacteria are known as bacilli (*singular,* bacillus).

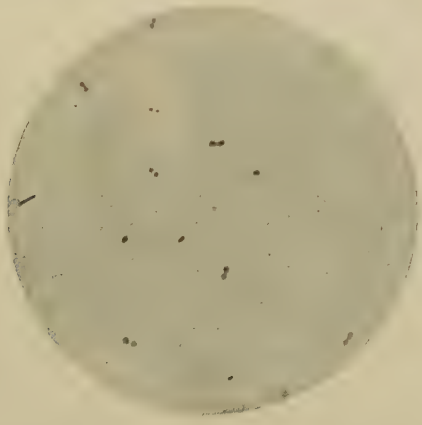


FIG. 17.—Diplococci. (*After Heinemann.*)

A second type of bacteria is the globular which may be likened to minute berries or at times to an egg. They are known as cocci (*singular,* coccus). Often when two remain connected

[illegible] an organism [illegible] The cocci may be large or small [illegible] themselves in various ways.

FIG. 18.—Streptococci. (*After Heinemann.*)

[illegible] spirilla (*singular*, spirillum). [illegible] The spiral may be loosely [illegible] There may be one, two, or more coils. At

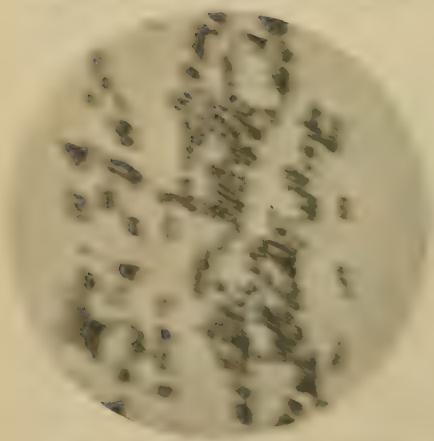


FIG. 20.—Spirilla. (*After Omilliansky.*)

[illegible] The curve may be so small and the curve so slight that [illegible] the microscope appears to be

Roughly speaking, there are about three time as many bacilli known as cocci and five time as many cocci as spirilla.

Variation in Form.—If one plants the Irish potato one expects to harvest Irish potatoes and not sweet potatoes. So it is with bacteria. If one plants bacilli, the bacilli and not the cocci will grow, or in other words the microscopic plants breed true just as do the higher plants. One may plant a potato and harvest

FIG. 21.—Involution forms of bacteria (enlarged about 1,000). 1, Bacillus proteus mirabilis. (*Hauser.*) 2, Bac. aceticus. (*Hauser.*) 3, Spirilla form of Bacillus anthracis. (*Petruschky.*) 4, Involution forms of Bac. halophilus. (*Russell.*) 5, Spirillum choleræ. (*van Ermengem.*)

potatoes which vary considerably in size and shape. The same is true in regard to bacteria. There may be a great variation within the same group. Some bacilli may reproduce club-shaped organisms many times larger than the average. This is characteristic of the bacteria which cause diphtheria, whereas the bacteria which grow in the nodules on the roots of alfalfa often take on the form of stars, crosses, and various grotesque

shapes. These so-called involution forms of bacteria have been likened to the lame and halt in human society and called degenerate forms of bacteria. This, however, is hardly an apt illustration, or a correct designation, for these peculiar shaped bacteria possess all the powers of the normal bacteria, and if they find their way into the body of an animal they are just as likely to produce their disease as are the normal microörganisms. Hence, we consider their changed shape as beng due to environmental conditions which have caused a change in shape, but which may not have affected their other properties and characteristics in the least.

Cell Structure.—Many bacterial cells are surrounded by a gummy mass called the capsule. In stained cultures this appears as a halo surrounding the bacteria. The presence of a great number of these capsulated bacteria in milk gives to it a ropy consistency. When this condition is present the outer coats of the bacteria stick together permitting the milk to be drawn out in long threads. When these capsulated forms get into flour or yeast they usually give to the bread a putty-like appearance.

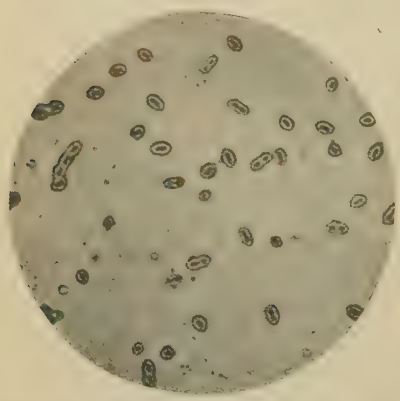


FIG. 22.—Capsulated bacteria. (*After Buerger.*)

There is a greater tendency for bacteria to form capsules when grown in body fluids than when grown on other media. The anthrax bacillus inoculated into the rat or lizard surrounds itself with a capsule, and hence, becomes very resistant. The capsule appears to be a defensive secretion and usually capsulated organisms are more likely to infect than similar non-capsulated organisms.

Bacteria have a cell membrane through which the food must pass and which retains the liquid of the cell within bounds. Lining the cell wall on the inner side is a dense layer of protoplasm which has a selective action on the food taken up by the cell. If the cell be placed in a strong sugar- or salt-solution, the cell shrinks and the ectoplasm is torn from the cell. This is known as plasmolysis.

In the cells of higher plants and animals is a distinct structure enclosed in its own membrane which stains deeper than the surrounding protoplasm and is known as the nucleus. Bacteria stain more uniformly throughout the whole cell, consequently it is a debated question whether bacteria in the true sense possess a nucleus, but some authorities teach that the greater part of the cell inclusion is nuclear material. Scattered throughout the cell may be seen dark granules. These bodies, termed metachromatic granules, may react to stains in such a way as to differentiate them from the rest of the cell and give us information as to their composition. They are probably the reserve food of the cell.

Protoplasm.—We have seen that it is a peculiarity of living matter to be always found in the organized form; that is, the body of the living organism is composed of organs, the organs of tissue, the tissue of cells. As we have seen, even the cell is composed of definite organized parts. This organized mass of living material composing the cell is spoken of as protoplasm. It is not, as was formerly believed, a definite chemical entity, but is composed of the various substances which enter into the vital construction of the cell. It in turn is organized. Just as the "Milky Way" of the heavens dissolves into individual stars when viewed through the telescope, so in a similar manner the cell protoplasm (highly magnified) shows organization within organization. Yes, it may be that it is not only organized but peculiar to the specific cell. All recognize that there are no two men exactly alike. The cattleman tells us that he has no two cattle alike; the sheepherder that there are no two sheep alike; the botanist that no two leaves or blades of grass are alike; and now the biologist tells us that the protoplasm composing our tissues is different from that composing the tissues of other individuals. Our individuality goes back to each individual cell. True, proteins are composed of the same amino-acids and protoplasm of the same proteins, fats, and carbohydrates, but these in turn are arranged in different orders. Calculating the theoretical number of permutations and combinations we find that there are countless billions of different protoplasms possible. These, while the stream of life is coursing through the living cell, are held in certain labile positions, but when death comes they swing back to the stable. Hence, it may be that in this sense individuality exists even amongst the bacteria.

Movement of Bacteria.—If one places a handful of hay in a bottle containing beef tea and allows it to stand in a warm place

for twenty-four hours and then examines a drop of the infusion in a hollow slide under the microscope, it will be found to be filled with living microörganisms. One notices that all of the particles within the drop are moving—even the smallest particles of hay. There is a great difference, however, in the way in which they move,—some swing back and forth, while others are seen to move swiftly across the field and out of sight. All of the bodies which are thus seen to move rapidly are microörganisms which have the power of movement. This action is known as vital movement.

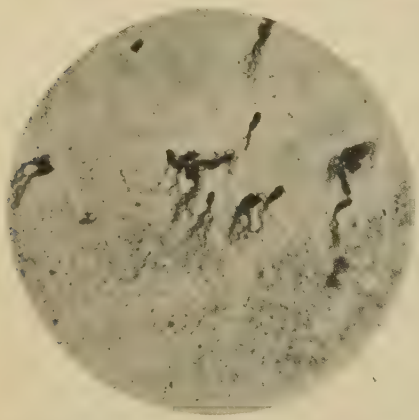


Fig. 23.—Bacteria with flagella on one pole. (*After Gruber.*)

By appropriate methods it has been shown that the motile bacteria have on their bodies long hair-like appendages called

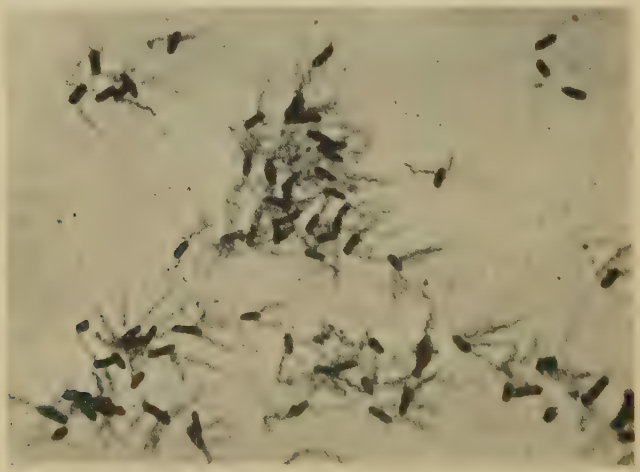


Fig. 24.—Bacteria with flagella on both poles. (*After Harrison.*)

flagella. These may be situated at one or both ends, or they may even surround the entire body of the organism. It is by the

striking of the water with these whip-like appendages that the organisms are able to move.

Bacterial movement shows great variation. It may be straight, or wiggling, or gyrating. Short rods often tumble about, appearing temporarily, when in an upright position, like small coccí. Spiral forms are whirling like corkscrews. The aspect becomes especially lively when in a drop of putrid liquid (old liquid manure, for instance) the motile bacteria are chased around by bacteria-hunting protozoa."

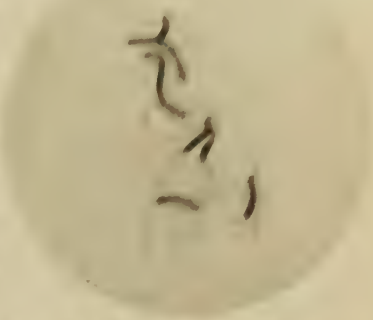


FIG. 23.—Bacteria with flagella, one showing fission. (After Migula.)

If one adds water to a bit of clay or a drop of Chinese ink and examines it in a hanging drop under the microscope, the particles will be seen in rapid motion. This fact was carefully observed and vividly portrayed for gold particles by the inventor of the ultra-microscope. "The tiny gold particles did not swing stationary in the water, they moved with astounding rapidity. He who has seen a swarm of flies dancing in the sunlight can obtain a notion of the movements of these tiny gold particles; they hop, dance, spring, crash together and fly asunder so rapidly that the eye can barely trace out their movements." This form of movement is manifested by all very small particles when in suspension. It is not due to life but results from the bombardment of the small solid particles by the molecules of the liquid. This was first described by the English botanist, Robert Brown, nearly a century ago, hence it is known as Brownian movement. Bacteria in common with all other small particles manifest Brownian movement. The bacteriologist has to learn to distinguish between it and the vital movements of bacterial cells.

As one examines moving bacteria under the microscope one would think that they were moving with the speed of an express train, but on actually measuring their speed it is found to be an illusion. The cholera organism has been known to attain for a short distance the enormous speed of eight inches an hour. That is, they travel in relation to size at about the same speed as does a running man.

Reproduction.—Bacteria quickly decompose the body of an ox. Plants and fruits quickly decay because of them. Millions of tons of organic matter are carried to rivers, lakes, and oceans each year. Bacteria change all of this into a residual ash and gaseous products. They modify the whole surface of the earth. One may wonder how it is possible for such small plants to accomplish such gigantic tasks. This is due to the rapidity of multiplication. The bacilli grow until they have reached a certain size, upon which they divide into two daughter cells. These in turn grow to maturity and likewise divide. At times the various cells on division may remain connected, and have

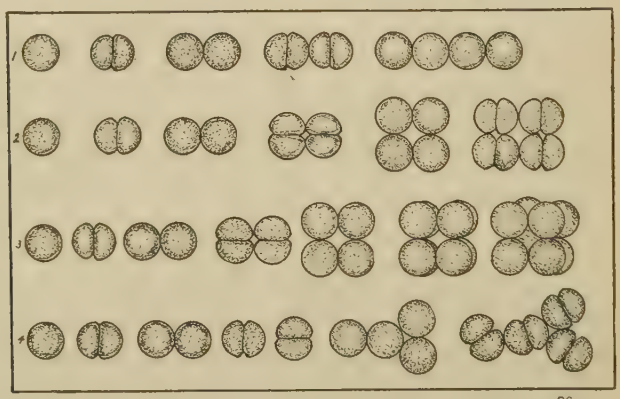


Fig. 26.—Forms of division and grouping in the cocci. 1, Streptococcus; 2, micrococcus with cells arranged in tetrads; 3, sarcina; 4, staphylococcus.

the appearance of a chain. Such a chain of organisms is called streptobacillus.

The cocci may divide in one, two, or three planes. When they divide in the same plane and the daughter cells touch at one side only and have the appearance of closely strung beads, they are known as streptococci. They may divide in one plane followed by division in another at right angles to the first, thus forming a sheet of cells. These are the micrococci. Others may divide in one plane followed by a second division which is not always at right angles so that the organisms form an aggregate which has the appearance of a bunch of grapes. These are the staphylococci. Still other organisms may divide in three planes,

thus giving rise to packets of cells—a condition similar to that which one would have if four marbles were placed on a level surface and four others placed upon them. These are the sarcina and have the appearance of a bale of cotton.

A bacterial generation is taken as the time required for a mature cell to divide and the resulting daughter cells to reach maturity. This process may be completed in one-half hour; at times, even more rapidly. Under less favorable circumstances it may be much longer. It has been estimated that if bacterial multiplication went on unchecked the descendants of one cell would in two days number 281,500,000,000 and in three days the descendants of this single cell would weigh 148,356,000 pounds! It has been further estimated by an eminent biologist that if proper conditions could be maintained for their life activity, in less than five days they would make a mass which would completely fill as much space as is occupied by all of the oceans on the earth's surface, considering the average depth of the water to be one mile.

Even in the face of these assumptions one need not fear, for bacteria have been on this earth and have been multiplying probably long before the advent of man, and as yet the earth has not been filled by them. This is due to there being a struggle among them just as there is among higher plants and animals. One knows that if wheat is sown too thick, none of it matures. Sometimes it is a lack of food, other times a lack of sunshine, and at still other times it is a lack of moisture which prevents its growth. So it is with bacteria. The food or water may give out; but, more often it is the bacterial products which have accumulated to the extent that prevent their multiplication.

Longevity of Bacteria.—Because of their method of multiplication there is no such condition as old age among bacteria, since both daughter cells are similar in age and composition. It is well-known that many bacteria while in the spore condition can survive for a quarter of a century. They have been obtained from soil which has been kept under air-dry conditions in fruit jars for over fifty years. Probably they may live for centuries.

Size.—The unit of measurement in microscopic work is the micron. It is one-thousandth of a millimeter in length, or approximately one-twenty-five-thousandth of an inch. It is represented by the symbol μ. Most bacteria are from 0.5 to 5 microns in length. Some are so small that they cannot be seen with the microscope. Others may be even 30 or 40 microns

in length. They are smallest in the case of the cocci and largest in the case of the spirilla.

Although there is a great variation in the size of bacteria, all are extremely small. Even the largest are not visible to the naked eye. The smallest are beyond the range of our most powerful microscopes. The Pfeiffer bacillus, the organism which was thought to cause influenza, is one of the smallest organisms known. It is a rod-shaped organism, and if placed end to end one hundred and twenty-five thousand of them would be required to make one linear inch. It would require fifteen thousand typhoid bacilli to reach an inch. The organism causing relapsing fever is one of the largest known, and of these some fifteen hundred would be necessary to reach one inch. We often magnify bacteria one thousand times, and then they appear as dots under the microscope. If we were to magnify man to this extent, he would be a giant indeed,—six thousand feet tall and fifteen hundred feet wide! In a sample of milk containing a billion bacteria in one cubic centimeter there is less than one-thousandth of its volume bacteria, while a little globe of bacteria no larger than a drop of water would consist of fifty billion bacteria.

Weight of Bacteria.—Knowing the size and density of bacteria, it is a comparatively simple matter to calculate their weight. Assuming the average bacterium to be a cylinder two microns long and 0.5 microns in diameter, the volume of such an organism would be 0.000,000,001,57 cubic millimeters. If it had a specific gravity of 1.2 its weight would be 0.000,000,001,884 milligrams, or in other words, it would require approximately five hundred thirty million of these to weigh a milligram, or thirty thousand time this number to weigh one ounce. It is the great number which makes them so effective. For example, one acre-foot of soil weighs 3,600,000 pounds. In each gram of soil there may be as many as 50,000,000 bacteria. This would mean that in one pound of soil there are 24,000,000,000 bacteria! From these figures we find that this is 6/1,325 of an ounce; therefore, the bacteria in an acre-foot of soil would weigh 1,026 pounds. That is, the minute bacteria which feed in an acre-foot of soil weigh approximately the same as the animal life that the plants growing upon an acre of pasture will maintain.

Spore Formation.—When adverse conditions arise many bacteria have the power of mobilizing the vital parts of their bodies into much smaller space than is occupied during their

normal life. In order to do this they exclude all excess moisture and surround themselves with a tough resistant coat. In some respects this form of the organism resembles the seed of the higher plants, and we speak of it as the spore. While in this stage the organisms can withstand many conditions which would quickly prove fatal to the growing or vegetative forms of bacteria. Some of them while in this condition can withstand the temperature of boiling water for many hours, or they may survive treatment with strong carbolic acid. For the time being they lose the power of multiplying, but they are still alive, and if brought into appropriate surroundings they will

FIG. 27.—Types of sporangia and spores in bacteria. 1, Spirillum with a terminal enlarged spore; 2, bacillus with a terminal enlarged spore; 3, bacillus with a terminal spore, not enlarged; 4, bacillus with an equatorial spore, not enlarged; 5, bacillus with two small terminal spores; 6, bacillus with two elongated spores; 7, 8, bacilli with equatorial swollen spores. (*After Buchanan.*)

change into normal bacteria just as the kernel of wheat changes into a young plant when placed into moist soil.

It is indeed fortunate for mankind that but few of the disease-producing organisms form spores. However, there are many of the bacteria such as those that cause fruit, meat, and various other food products to spoil, which do form very resistant spores. That is why many food products have to be heated for such a long time or to such high temperatures to make them keep. At times, in order to avoid heating the substances to a high temperature, the intermittent method of sterilization is made use of. In this method the substance which is to be sterilized is heated to 100° C. for a short time. This heating kills all of the organisms which are not in the spore form. After standing in a moderately warm place for twenty-four hours, during which time the spores vegetate, it is again heated for a short time, thus killing the others which have started to grow.

Often this is repeated even a third time before the vegetables or other products are canned.

The manner of formation of the spore within the body of the organism is intensely interesting, for it varies with different species. The bacteriologist has devised means whereby he can color the body of the organism one color and the spore within the body a different color. In some instances if we stain the various organisms in the first stages of spore formation, we find the little red dot situated within the center of the blue body. In others, it is also in the center, much wider than the body of the organism; hence, we have a boat-shaped organism or clostridium. In still other bacteria the spore forms at the end, and we have the drumstick-appearing organism, or capitate.

FIG. 28.—Illustrating spore germination.

This is the case with the organism causing lockjaw. Spores when brought into appropriate conditions germinate. The process of germination varies in different bacteria.

Morphology of Yeasts.—Yeasts vary in form from a sphere to an ellipsoid. They are surrounded with a rather thick membrane in which is a colorless liquid containing vacuoles and granules. On staining, they are found to possess a definitely organized nucleus. Most yeasts are considerably larger than the bacteria. Their characteristic method of multiplication is by budding. The bud first appears as a small protuberance separated from the mother cell by a very narrow collar, and increases in magnitude until it has acquired a certain size upon which it separates from the mother cell. The daughter cell grows rapidly and soon equals the mother cell in size, after which it in turn forms buds. When multiplication is very active, each cell may form many buds on different parts of its surface at the same time. It not infrequently happens that

buds while still attached to the mother cell begin to form on themselves buds. This gives rise to a small colony of cells.

Yeasts produce spores which tide them over adverse conditions, but their spores differ from those of the bacteria in that a single yeast cell may give rise to a number of spores; hence, this becomes a process of multiplication.

The yeasts ferment sugars with the formation of alcohol and carbon dioxide and are often classified according to the specific sugars which they ferment. Few of the yeasts produce disease in man or the lower animals. However, they have been used to combat in man certain bacterial infections such as boils and

FIG. 29.—Yeast cells showing budding. (*After Van Hest.*)

carbuncles. Their action is probably due to the vitamin which they contain, as well as to the fact that they increase the activity of the defensive substances, particularly of the white corpuscles of the blood, toward the bacteria which are invading the body of the individual suffering with boils.

Molds.—The body of a mold is composed of numerous cells, whereas the bodies of bacteria and yeasts are single-celled. The thread-like processes which compose their bodies are known as *mycelia*. The individual threads are called *hyphae*. In the molds there is a division of labor. Some threads serve for the taking up of nutrients and are called vegetative hyphae; others produce spores and are called fertile hyphae. The cells composing the molds are similar to those of yeasts in that they have a definite nucleus. Molds multiply mainly by the production of spores which are produced in the fertile hyphae above the

surface of the medium; hence, they can be dislodged and spread by currents of air. Molds grow under conditions of acidity and moisture which are inimical to the growth of bacteria and yeasts. They differ from bacteria in that they all require free

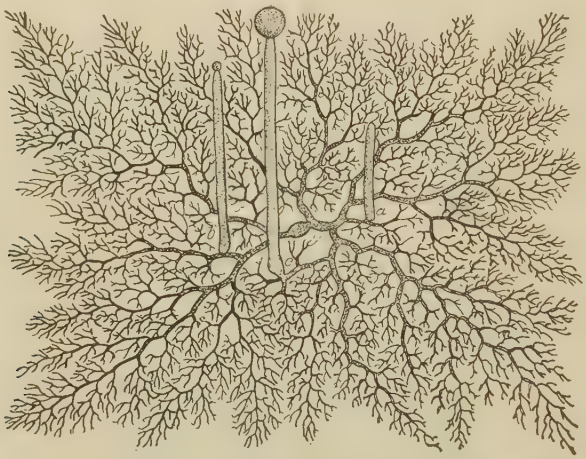


FIG. 30.—A mold (mucor mucedo) single-celled mycelium with three hyphae and one developing sporangium. (*After Kny, from Tavel.*)

oxygen. They play a great part in the spoilage of foods and are second only to bacteria in importance in soil processes. Few molds are pathogenic, but they produce diseases in man and the lower animals to a much greater degree than do yeasts.

CHAPTER VII

CLASSIFICATION OF BACTERIA

Organization is essential to life. The lack of it is death. Classification is a primary requisite to efficiency and progress in human activity, and as soon as it is absent the result is chaos. Even in the infancy of the race man endeavored to organize and classify the things with which he dealt. In most forms of modern endeavor the materials with which man deals are so numerous and complex that organization and classification becomes not only valuable but absolutely essential to progress. The astronomer classifies the stars, and the chemist the numerous compounds with which he works. Likewise, the biologist must classify the plants and animals with which he works.

Functions of Classification.—With the aid of a careful system of classification the librarian can quickly locate any volume in the library. By a different system the police officer is at once able to tell whether the finger prints before him are those of one of the millions of prints which are on file in the country. Thus, the aim of classification in any science is to group things in such a way as to bring out their similarities and differences. All individuals in the world could be classified according to finger prints. First, they could be divided into five great divisions according to finger-print patterns—arches, loops, groups, whorls, and central pockets. Those belonging to the arches could then be divided into seven classes—natural, dotted, tented, approximating loops, transitional patterns, staircase arches, and irregular arches; and each of these in turn could be further subdivided until even with such a complex aggregate as the finger prints of humanity a few very similar ones could be placed together, and what at first appeared a bewildering mass of data becomes an organized workable whole. The function of classification then may be summarized according to Huxley's definition as modified by Jevon's as follows:

"By the classification of any series of objects is meant the actual or ideal arrangement together of those things which are like and the separation of those things which are unlike, the

purpose of the arrangement being primarily, to disclose the correlations or laws of union of properties and circumstances and secondarily, to facilitate the operations of the mind in clearly conceiving and retaining in memory the characters of the objects in question."

Method of Classification.—Classification brings out relationships. This is accomplished by arranging groups within groups. It is used in all our political and religious organizations, but probably reaches its highest state of perfection in the army. An army is a specific organized system which is composed of definite organized units, each unit of which is nicely fitted into the whole. That is, an army is composed of divisions; each division is composed of brigades; each brigade of regiments; each regiment of batallions; each batallion of companies; each company of platoons; and each platoon of squads. In such an organization each individual occupies a definite specific place. A somewhat similar arrangement is used in classifying the 250,000 species of plants which have been studied. We have seen that the plant kingdom is divided into four great divisions. Each of these divisions is composed of classes; each class is composed of orders; each order is composed of families; each family is composed of tribes; each tribe is composed of genera; and each genus is composed of species. Within the species there may be different races or strains. In a system such as this each plant has a specific place in the classification.

The division in which we are interested, the Thallophytes, is composed of two classes: (1) The algae which possess the green pigment, chlorophyll, and are, therefore, able to manufacture their own food. They include all the seaweeds and their fresh-water allies. (2) The fungi which lack chlorophyll and, consequently, are obliged to obtain their food from living animals and plants, or from dead organic material. To this group belongs that vast array of organisms—the bacteria, molds, yeasts, blights, rusts, toadstools, mushrooms, and similar plants. The order Eubacteria or true bacteria, one of the subdivisions of the general class is divided into families, these into tribes, and so on. But before following these subdivisions further it is well to consider briefly the factors used in the classification of bacteria and the method of naming them.

Nomenclature.—In the early history of botany the worker, in referring to a species of plant, used a descriptive phrase. Another worker might use a different phrase to describe the same

plant. This resulted in confusion, for it was often impossible to determine whether both writers were referring to the same or to different plants. To overcome this difficulty the great Swedish naturalist, Linnaeus, devised the Binomial System of naming plants, so called because each species is given two names. The first is the name of the genus and the second the name of the species. The first is common to many; the second name is specific. The method is similar to that used in naming individuals. The surname is common to the family; the given name is specific. However, it will be noted in botanical nomenclature that the specific name comes last, or, in other words, it is the reverse of what it is in family names. Therefore in botanical work one gives first the name of the genus which must be capitalized and which may be written in full or abbreviated. This is followed by the specific name of the species. The name of the species is written in full and with a small letter. Usage at times sanctions the use of a capital where the name is a proper noun. However, even then it is better to use a small letter. In order to avoid confusion and to make more clear what specific plant is referred to there is often placed after the name of the species the name or abbreviation of the name of the botanist who first used the name for the species. Using this system, the name for wheat would be written *Triticum sativum* or *T. sativum*. That for the potato is written *Solanum tuberosum* or *S. tuberosum*. Unfortunately, this system is applied less satisfactorily with regard the microörganisms.

Difficulties in Classifying Bacteria.—The difficulties inherent in the classification of bacteria are numerous. (1) The small, simple structure of the microörganisms makes it impossible to work out a satisfactory classification on a purely morphological basis as in the case of the higher plants. (2) Many physiological characteristics, such as pigment production which at first sight may appear useful, are not constant and, hence, cannot be used. (3) Our knowledge of the characters and make up of the bacteria even at the present time is far from complete. (4) Bacteria play a part in many fields of activity, and consequently the criteria whereby they are recognized vary greatly according to the art or science in which they are studied. For agricultural and industrial purposes it may be more practical and quite sufficient to classify bacteria according to their activity. For example, we may classify them as butyric- or lactic-acid-producing, ammonifying, nitrifying, or nitrogen-fixing organisms. (5)

There has been a great tendency on the part of many authors to apply whole descriptions to microörganisms instead of using the binomial names as agreed to in botany. For instance, the following cumbersome unscientific designation has been used: *Micrococcus acidi paralactici liquefaciens Halensi* or *Granulobacillus saccharobutyrecus immobilis liquefaciens.*

Migula's Classification of Bacteria.—Numerous attempts have been made to classify bacteria, but none are without flaws. One of the most extensively used throughout America is the one proposed about thirty two years ago by Migula. Migula makes use of morphology, and groups cells according to method of multiplication and motility.

MIGULA'S CLASSIFICATION

Bacteria (Schizomycetes).—Fission fungi (chlorphyl free), cell division in one, two, or three directions of space. Many varieties possess power of forming endospores. Whenever motility is present, it is due to flagella, or more rarely, to undulating membranes.

FAMILY I. COCCACEAE.—Cells in free state spherical. Division in one, two, or three directions of space, by which each cell divides into two, four, or eight segments, each of which again develops into a sphere. Endospore formation rare.

Genus I. Streptococcus.—Cells divide in one direction of space only, for which reason, if they remain connected after fission, bead-like chains may be formed. No organs of locomotion.

Genus II. Micrococcus (Staphylococcus).—Cells divide in two directions of space, whereby, after fission, tetrad and grape-like clusters may be formed. No organs of locomotion.

Genus III. Sarcina.—Cells divide in three directions of space, whereby, after fission, bale-like packets are formed. No organs of locomotion.

Genus IV. Planococcus.—Cells divide in two directions of space, as in micrococcus, but possess flagella.

Genus V. Planosarcina.—Cells divide in three directions of space as in sarcina, but possess flagella.

FAMILY II. BACTERIACEAE.—Cells long or short, cylindrical, straight, never spiral. Division in one direction of space only, after preliminary elongation of rods.

Genus I. Bacterium.—Cells without flagella, often with endospores.

Genus II. Bacillus.—Cells with peritrichal flagella, often with endospores.

Genus III. Pseudomonas.—Cells with polar flagella. Endospores occur in a few species, but are rare.

FAMILY III. SPIRILLACEAE.—Cells spirally curved or representing a part of a spiral curve. Division in one direction of space only, after preceding elongation of cell.

Genus I. Spirosoma.—Cells without organs of locomotion. Rigid.

Genus II. Microspira.—Cells rigid, with one or, more rarely, two or three polar undulated flagella.

GENUS III. SPIRILLUM.—Cells rigid, with polar tufts of five to twenty flagella usually curved in semicircular or flatly undulating curves.

GENUS IV. SPIROCHAETA.—Cells sinuously flexible. Organs of locomotion unknown, perhaps a marginal undulating membrane.

FAMILY IV. CHLAMYDOBACTERIACEAE.—Forms of varying stages of evolution, all possessing a rigid sheath (Hülle), which surrounds the cells. Cells united in branched or unbranched threads.

GENUS I. STREPTOTHRIX.—Cells united in simple, unbranched threads. Division in one direction of space only. Reproduction by non-motile conidia.

GENUS II. CLADOTHRIX.—Cells united or pseudodichotomously branching treads. Division in one direction of space only. Vegetative multiplication by separation of entire branches. Reproduction by swarming forms with polar flagella.

GENUS III. CRENOTHRIX.—Cells united in unbranched threads, at first with division in one direction of space only. Later the cells divide in all three directions of space. The daughter-cells become rounded and develop into reproductive cells.

GENUS IV. PHRAGMIDIOTHRIX.—Cells at first united in unbranched threads, dividing in three directions of space, thus forming a rope of cells. Later some of the cells may penetrate through sheath, and thus give rise to branches.

GENUS V. THIOTHRIX.—Unbranched, non-motile threads, inclosed in fine sheaths. Division of cells in one direction only. Cells contain sulphur granules.

FAMILY V. BEGGIATOACEAE.—Cells united in sheathless threads. Division in one direction of space only. Motility by undulating membrane as in Oscillaria.

GENUS BEGGIATOA.—Cells with sulphur granules.

This classification is especially unsatisfactory to the student of agricultural bacteriology as it is hard to place some of the soil organisms and plant pathogens in the classification. Moreover, the small number of genera makes it necessary to class together organisms which are morphologically similar but which possess marked differences physiologically.

Classification Society of American Bacteriologists.—Many attempts have been made to devise a classification which will overcome these difficulties. In 1909 Orla-Jensen, a Danish bacteriologist, proposed a system in which biochemical properties were used. He coined names suggestive of the activity of the microörganism. Recently a committee of the Society of American Bacteriologists formulated a method in which both the morphology and physiology of the organisms are made use of in their classification. Moreover, the method of naming is that followed by the botanists. The important points can be summarized as follows: (1) The name should be a binomial and not a

trinomial one. (2) Latin names should be used for all groups, and with certain limitations the oldest designation for each organism or group is to be the preferable one. (3) In naming, orders are designated with the ending *ales*, families with the ending *aceae*, sub-families with *oideae*, tribes with *eae*, and subtribes with *inae*. (4) The generic name is written with a capital.

The families and genera of the bacteria, as reported by the committee, are as follows:

THE CLASSIFICATION OF BACTERIA ACCORDING TO BERGEY ET AL. (1923)

Key to the Orders of the Class Schizomycetes

1. Simple and undifferentiated forms, the true bacteria ..... Eubacteriales
2. Specialized or differentiated forms.
   - a. Plant like.
     - b. Mold like ..... Actinomycetales
     - bb. Not mold like.
       - c. Sheathed ..... Chlamydobacteriales
       - cc. Not sheathed.
         - d. Sulphur bacteria ..... Thiobacteriales
         - dd. Slime-mold like ..... Myxobacteriales
   - aa. Protozoa like ..... Spirochaetales

Key to the Families of the Order Eubacteriales

1. Organisms obligate aerobes, using oxygen for direct oxidation of carbon, hydrogen, or nitrogen or compounds of these. Cells usually rod-shaped, occasionally spherical ..... Nitrobacteriaceae
2. Not securing growth energy as under I.
   - a. Cells spherical ..... Coccaceae
   - aa. Cells not spherical.
     - b. Cells spiral ..... Spirillaceae
     - bb. Cells straight rods.
       - c. Not producing endospores. Cells motile or non-motile ..... Bacteriaceae
       - cc. Producing endospores ..... Bacillaceae

Key to the Tribes and Genera of the Family Nitrobacteriaceae

1. Organisms oxidize simple compounds of carbon and nitrogen ..... Nitrobactereae
   - a. Cells capable of securing growth energy by the oxidation of hydrogen to form water ..... Hydrogenomonas
   - b. Cells oxidize methane to form CO_2 and water .. Methanomonas
   - c. Cells oxidize CO to form CO_2 ..... Carboxydomonas
   - d. Cells oxidize ammonia to form nitrites ..... Nitrosomonas

e. Cells oxidize nitrites to nitrates..... Nitrobacter
f. Cells oxidize alcohol to form acetic acid..... Acetobacter
g. Cells oxidize compounds of sulphur..... Thiobacillus
2. Organisms capable of fixing free nitrogen of the air Azotobactereae
a. Cells capable of fixing free atmospheric nitrogen when growing in solutions of carbohydrates Azotobacter
b. Cells capable of fixing free nitrogen when growing symbiotically on the roots of Leguminosae Rhizobium

Key to the Tribes of the Family Coccaceae

1. Strict parasites, failing to grow, or growing very poorly on usual artificial media. Cells normally in pairs, occasionally in tetrads..... Neissereae
2. Parasites (except Leuconostoc) growing best in media containing serum. Occur in pairs of chains..... Streptococceae
3. Facultative parasites or saprophytes. Cell aggregates of groups, packets or zoogleal masses. Growth abundant..... Micrococceae

Key tothe Genera of the Tribe Neissereae

One genus only: Neisseria

Key to the Genera of the Tribe Streptococceae

1. Parasites growing poorly, or not at all, on artificial media. Cells usually in pairs..... Diplococcus
2. Saprophytes, usually growing in cane sugar solutions. Cells in pairs or chains..... Leuconostoc
3. Chiefly parasites. Normally forming short or long chains, sometimes pairs, but never packets..... Streptococcus
4. Parasites. Cells in groups or short chains, rarely in packets..... Staphylococcus

Key to the Genera of the Tribe Micrococceae

1. Facultative parasites or saprophytes. Cells in plates or irregular masses (never in long chains or in packets)..... Micrococcus
2. Cell division occurs in three planes forming packets Sarcina
3. Saprophytes. Cells in groups or packets. Form red pigment on agar..... Rhodococcus

Key to the Genera of the Family Spirillaceae

1. Cells short, bent rods, rigid, single or united into spirals..... Vibrio
2. Cells rigid, of various thickness and length and pitch of the spiral, forming either long curves or portions of a turn..... Spirillum

Key to the Tribes of the Family Bacteriaceae

1. Produce pigment on solid media. The pigment may be red, yellow, violet, green, or blue	Chromobactereae
2. Without pigment formation on agar or gelatin	Achromobactereae
3. Cellulose digesting organisms occurring in soil	Cellulomonadeae
4. Plant pathogens, growth usually whitish	Erwineae
5. Gram-positive rods, growing freely on artificial media. Do not attack carbohydrates	Zopfeae
6. Gram-positive rods growing freely on artificial media. Generally act on carbohydrates with formation of acid and gas.	
a. Without capsules	Bactereae
7. b. Encapsulated	Encapsulateae
8. Rods often long and slender. Gram-positive. Non-motile. Usually produce lactic acid from carbohydrates	Lactobacilleae
9. Anaerobic, non-spore-forming rods	Bacteroideae
10. Gram-negative rods, showing bipolar staining. Parasitic forms	Pasteurelleae
11. Minute parasitic forms growing only (or best) in the presence of hemoglobin, ascitic fluid or other body fluids	Hemophileae

Key to the Genera of the Tribe Chromobactereae

1. Small, aerobic rods, producing a red or pink pigment on agar or gelatin	Serratia
2. Small, aerobic rods, producing a yellow pigment on gelatin or agar	Flavobacterium
3. Small, areobic rods, producing a violet pigment on solid media	Chromobacterium
4. Small, aerobic rods, producing a green or blue-green pigment	Pseudomonas

Key to the Genera of the Tribe Achromobactereae

One genus only: Achromobacter.

Key to the Genera of the Tribe Cellulomonadeae

One genus only: Cellulomonas.

Key to the Genera of the Tribe Erwineae

1. Motile rods.	
a. Flagella peritrichous	Erwinia
2. Rods motile or non-motile. Motile forms possess polar flagella	Phytomonas

Key to the Genera of the Tribe Zopfeae

One genus only: Zopfius.

Key to the Genera of the Tribe Bactereae

A. Ferment dextrose with production of acid, or acid and gas.
  1. Gas formed from dextrose
    a. Gas formed from lactose
      b. Acetyl-methyl-carbinol not formed from dextrose..... Escherichia
      bb. Acetyl-methyl-carbinol formed..... Aerobacter
    aa. Gas not formed from lactose.
      b. Gas formed from sucrose..... Proteus
      bb. Gas not formed from sucrose..... Salmonella
  2. Gas not formed from dextrose.
    a. Acid formed from dextrose..... Eberthella

AA. Do not form acid or gas from any of the carbohydrates..... Alcaligenes

Key to the Genera of the Tribe Encapsulateae

One Genus only: Encapsulatus

Key to the Genera of the Tribe Lactobacilleae

One Genus only: Lactobacillus.

Key to the Genera of the Tribe Bacteroideae

One Genus only: Bacteroides.

Key to the Genera of the Tribe Pasteurelleae

One Genus only: Pasteurella.

Key to the Genera of the Tribe Hemophileae

1. Aerobic species..... Hemophilus
2. Anaerobic species..... Dialister

Key to the Genera of the Family Bacillaceae

A. Aerobic forms, mostly saprophytes..... Bacillus
B. Anerobic forms, Often parasitic..... Clostridium

CHAPTER VIII

STUDYING BACTERIA

Bacteria are ubiquitous; they are extremely small; and many of them are very resistant to external influences. The early workers in the field of bacteriology did not possess this information; hence, when bacteria were first discovered, it was only natural to conclude that they were spontaneously produced. This idea was overthrown only when man learned how to free his materials from these minute specks of life and to keep them free from external life. It was not until he had devised means for growing these microörganisms that he learned their properties and characteristics. Their minute structure rendered useless the methods used to study the higher forms of life; so, it was necessary to devise technique peculiar to the specific subject. For freeing his materials from bacteria, man soon learned to use heat and chemicals; to keep them free, cotton; to grow them at will, certain foods; and to study their shape and structure, he used the microscope. He learned their action by studying the changes produced in the living and dead materials into which he placed them.

Sterilization.—Materials free from microörganisms are said to be sterile. The process by which they are rendered free is called sterilization. It is absolutely requisite that the worker in bacteriology have quick, easy, and effective methods of sterilizing the things with which he deals; otherwise, it would be impossible for him to gain specific information concerning any microörganism, as the ones which he would find in his medium would be not only those placed there by himself but all others which chanced to be in his materials. We have seen that it was this very principle which played such a tremendous part in the controversy on spontaneous generation.

Sterilization may be effected by either chemical or physical means. We shall consider here the physical processes, leaving the chemical methods for a later chapter. The physical methods in general use are heat, light, and filtration.

Sterilization by Heat.—The most general method of sterilization is by means of heat. It is cheap, effective, and readily applied.

It is used in various ways, depending upon the nature of the substance to be sterilized. The following are the principal methods used by the bacteriologist: (1) flame, (2) dry heat, (3) boiling water, (4) streaming steam, and (5) steam under pressure.

The Flame.—The platinum needle, tweezers, and various other instruments used by the bacteriologist in handling infected material can be quickly and effectively sterilized by flaming. In this, certain precautions are necessary: (1) All parts of the object infected must be heated to a temperature high enough to destroy all forms of life. (2) Where the infected object is

a b c d

FIG. 31.—Sterilizers. (a) Hot air sterilizers; (b) upright autoclaves; (c) steam sterilizers, (d) horizontal autoclave.

covered with moisture, care must be taken not to heat too quickly; otherwise, spattering may occur. This may scatter the infected material about, and if pathogens are present dire results may follow.

Valueless infected objects may be burned. The sputum of individuals ill with communicable diseases may be received in clean rags and then burned, thus preventing the infecting of others. This used to be the principal method employed in protecting man against infection.

Hot Air.—Many materials are too valuable to burn; hence, one must turn to other effective but less destructive methods of sterilization. Petri dishes, test tubes, and many other forms of glassware may be effectively sterilized by heating in an oven for one hour at a temperature of 160° C. This is the temperature at which cotton just begins to char. Its effectiveness depends

upon the charring of the bodies of the microbes. Although the common kitchen oven can be effectively used for this purpose, there are many easily operated gas or electric hot-air sterilizers on the market.

Boiling Water.—Forceps, scissors, knives, hypodermic needles, and many other objects may be readily freed of infection by boiling for ten minutes in water containing 1 per cent of soda. This is also an effective method for cleaning milk bottles, buckets, and other utensils.

Sterilization by Streaming Steam.—Moist heat is more effective in destroying microörganisms than is dry. This is due to a number of causes: (1) Moist heat is more penetrating. (2) Life is destroyed when the protein composing the body is coagulated, and the temperature at which coagulation occurs depends upon the moisture present. Dry proteins can be heated to the charring point without coagulation. (3) Hot steam penetrates the various substances being sterilized, and on condensing liberates great quantities of heat which in turn penetrate all of the material; hence, the favorite method of sterilization is by means of steam. This, as generated, is known as streaming or live steam. One of the best methods of generating it for laboratory use is by means of an Arnold Steam Sterilizer. This is provided with a false bottom so that the steam passes up through the materials to be sterilized, and as the steam condenses the water is returned to the receptacle to be reheated. It should be provided with an opening in the top into which a thermometer can be fitted, in order that the worker may be certain of the temperature. Test tubes should be placed in wire baskets; other materials should be loosely packed so the steam can penetrate all. The temperature (100° C.) reached by streaming steam at sea level is sufficient to kill the non-spore-bearing organisms in 15 minutes. Some resistant spores will withstand this treatment for hours. To obviate this difficulty, either the discontinuous method or steam under pressure is used.

Discontinuous or Intermittent Sterilization.—Discontinuous boiling as a means of sterilization was introduced in 1877 by Tyndall, who made the following remark concerning it: "Five minutes of discontinuous heating can accomplish more than five hours' continuous heating." This method takes advantage of the fact that all bacteria, while in the vegetative stage, are quickly killed by live steam but are very resistant when in the resting or spore stage. The material to be sterilized is heated

in steam or boiling water from 15 to 60 minutes, depending upon the nature and size of the container. In sterilizing agar, time is counted from the point at which the agar melts. Large containers require longer for the heat to penetrate than do small ones. After the material has been heated the necessary length of time, it is set aside for 24 hours in a comparatively warm place. During this time the spores germinate. The material is then reheated, allowed to stand a second time, and after 24 hours reheated again. This process may be repeated a third or even a fourth time. This method is useful and effective for sterilizing media containing sugars or body fluids which would be changed by higher temperatures. It is often used by the housewife in the processing of fruits and vegetables. However, when the spores do not germinate between heatings it often fails. A failure of germination may be due to a number of causes: (1) The medium may be unsuited for the growth of some particular organisms present. (2) The material may have been over-heated in the first process, and the germs retarded to such an extent that they will not germinate in the time allotted. (3) There may be present anaërobes which will not germinate in the media until the free oxygen has been removed. Such cases can be handled by heating to a higher temperature in steam under pressure. This is done in an autoclave.

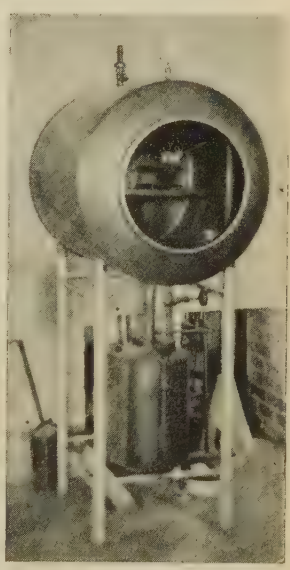


FIG. 32.—Autoclave.

Autoclave Sterilization.—Bacteria are killed by boiling water or live steam, but some spores resist these treatments for hours. Materials containing such spores may be sterilized by exposing for a time to a higher temperature. The temperature and time necessary depend on the nature of the material to be sterilized, as well as on the microörganisms present. Agar media in test tubes are readily sterilized by exposing 15 minutes to a temperature of 120° C. Larger containers require a longer time. The necessary temperature is readily obtained by heating in an autoclave. This is a cylindrical vessel in which the steam is generated or forced in at the required temperature. It is provided with a

pet cock through which the steam may be permitted to escape, a pressure gauge, and often a thermometer. There is also a safety-valve which can be set to slowly release the steam at any desired pressure. Certain precautions are necessary; otherwise, loss of media, injury to autoclave, or other accidents may result. (1) Care should always be taken to see that sufficient water is in the autoclave to carry it through the time it is being heated, for should it go dry it will be burnt out and ruined. (2) Where test tubes in baskets are placed directly on top of each other there is a great tendency for the water to condense and run down into the under tubes, thus, wetting the cotton stoppers and spoiling the media. (3) The steam should be allowed to issue from the opening several minutes before closing; otherwise, pockets of air are produced around the materials to be sterilized. This results in the pressure gauge giving a false reading in so far as temperature is concerned. (4) Time should be counted from the point at which the required pressure is reached. Agar should be in the liquid form when placed in the autoclave (or else a correspondingly longer time allowed for sterilization). (5) The temperature should be allowed to fall below the boiling point before the autoclave is opened; otherwise, the plugs may be forced from the containers, or the contents themselves boil out.

Culture media (with the exception of those containing some of the carbohydrates or body fluids) may be sterilized by this means. Where gelatin is to be sterilized in the autoclave, the pressure should not exceed 10 pounds for 5 minutes, and the media quickly cooled in the ice box. If this is not done the gelatin will fail to solidify.

The autoclave is extensively used in the processing of various food products as well as in the sterilizing of infected bedding and clothing.

Sterilization by Filtration.—Air and other gases are readily sterilized by drawing through cotton. This method is used where sterile air is required. Bacteriological media are readily preserved by stoppering the container with cotton and then sterilizing. However, if the stoppers become moist, or if considerable time elapses, certain microörganisms tend to grow through the cotton plugs.

Various types of filters made of unglazed porcelain or compressed diatomaceous earth are used where the application of heat will injure the solution, as in the case of certain sugars,

tissue extracts, and serums. These filters are made in various grades of fineness; hence, they are sometimes used in separating organisms of varying sizes. Those forms of organisms which pass through the filter are spoken of as filterable viruses. The filter itself and accompanying parts are at first sterilized in the autoclave, after which the material to be filtered is passed through. The filter can be cleaned by passing through it considerable clear water. Where the pores become clogged the filter can often be revived by heating; but in this case, care must be used to see

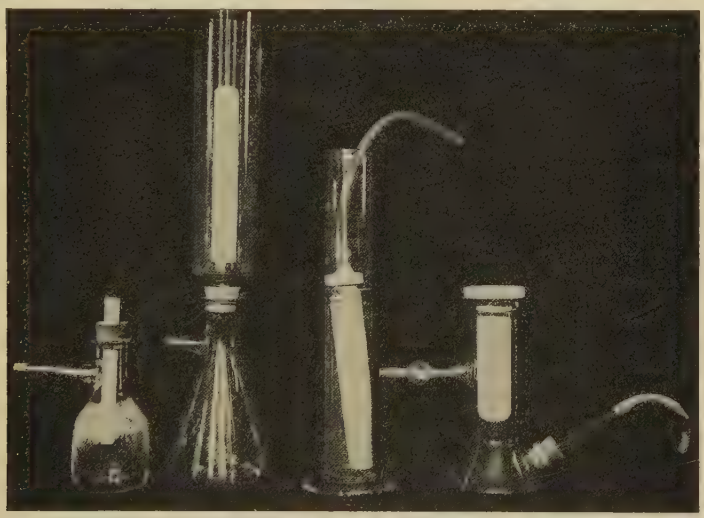


FIG. 33.—Different types of bacteriological filters: *a*, Kitasato; *b*, Berkefeld; *c*, Chamberland; *d*, Reichel. (*From McFarland Pathogenic Bacteria and Protozoa.*)

that the heat has not produced small fissures which cause the filter to leak.

Coarse filtration is sometimes accomplished by the use of paper, pulp, or sand. The latter is often used in the filtration of city water supplies.

Culture Media.—All nutrient materials used in the laboratory for the growth of microörganisms are known as *cultural media.* They are essential for the keeping, growing, and the studying of the cultural characteristics of microörganisms. They are variously classified as synthetic and non-synthetic; liquid and solid; liquefiable and non-liquefiable.

Synthetic media are those composed of pure chemicals and of known composition. They are valuable in the work for which they are used because they can be accurately duplicated by other workers. The goal towards which the bacteriologist strives is the preparation of synthetic media on which he can grow any of the microörganisms. This is far from possible at the present time; hence, he uses non-synthetic media, such as potatoes, milk, beef tea, blood, and other natural products.

A nutrient medium suitable for the growth of microörganisms must possess the following characteristics: (1) It must contain nutrients suitable for the organism that one desires to grow.

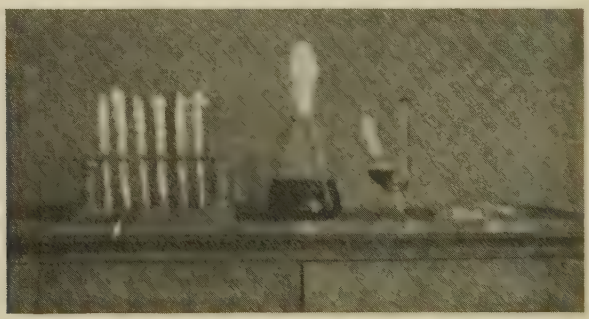


FIG. 34.—Apparatus used in the growth of bacteria. 1, Test tube cultures of bacteria; 2, Erlenmeyer flask of bouillon; 3, fermentation tubes containing bouillon; 4, Petri dish.

This may be a simple salt solution, as is used with some soil bacteria, or it may be a complex body tissue or fluid, as is the case with many of the pathogens. (2) It must possess a suitable reaction. Most bacteria require a neutral or slightly alkaline medium; whereas, many molds flourish in an acid medium. (3) The nutrient media must be sterile. The method used in its sterilization depends upon the specific medium.

One of the principal liquid media is beef tea. When small quantities of the seaweed, agar-agar, are added to this, we obtain a solid medium. If gelatin is added in place of agar-agar, there results a medium which is solid but which can be liquefied by certain organisms. The nature of the growth and changes produced in the various media are used by the bacteriologist in the characterizing and identifying of bacterial species.

Cultures of Bacteria.- A culture is any growth of organisms on laboratory media. A colony is such a growth that has originated

from a single organism. A culture in which only one species of organisms is present is called a pure culture; one in which more than one is present is mixed. As bacteria occur in nature, they are usually mixed. In order to study their specific properties, workers find it necessary to obtain them as pure cultures; hence, we shall consider very briefly, the methods of obtaining pure cultures.

Pure Cultures. Early workers used the so-called dilution method for obtaining pure cultures of microörganisms. It consists in adding a small quantity of the material to be studied to a sterile liquid medium. A small quantity of this, in turn, is transferred to a second portion of sterile media, and this process is repeated a number of times. The inoculated media is then incubated until growth appears on the more heavily seeded media. If the dilutions are continued far enough, some of the tubes will show no growth. Some of the tubes receiving only small quantities of the material may have received only a single organism, and hence, these would be pure cultures. This, however, could be determined only by further microscopic and cultural studies. It is evident that such a method is based on the expectation that some tube is to receive only one species. Furthermore, the method is long and cumbersome and has been practically replaced by later and more specific methods.

Plate Method.—A great advancement was made in the obtaining of pure cultures when Koch introduced the use of solid media. In general, this method is as follows: Tubes of gelatin or agar media are liquefied and then cooled to 42° C. The material from which one wishes to obtain pure cultures is transferred to the liquefied tubes by means of sterile platinum wire. A loopful of the material is placed into a tube of the medium and thoroughly mixed with the medium by tilting and rolling the tube, care being taken not to produce bubbles. From the well-mixed tube three loops are carried over to a second tube which is mixed in the same manner. From the second tube five loops are carried to a third tube and mixed. The lips of the tubes are flamed and the contents poured into sterile Petri dishes. During the pouring the cover of the Petri dish is raised just sufficiently to permit pouring, the lid acting as a cover to prevent dust entering the plate. The dish is then agitated back and forth, so that the media is evenly distributed over the surface. The plates are incubated at the optimum temperature until colonies appear on the surface. It is well to invert agar plates while incubating, thus preventing

the collection of moisture on the surface of the medium which would cause the colonies to run together.

When growth appears on the media it is examined by the low-power of the microscope or a hand lens. Those colonies which stand out free from others and which have a characteristic homogenous appearance are selected for further study. This is done by fishing out the isolated colonies with a platinum needle and studying them microscopically and culturally.

FIG. 35.—Dilutions made in Petri dishes.

Microscopic Study.—Microörganisms can seldom be identified by the microscope alone. It will, however, reveal certain characteristics, such as individual form, arrangement, reaction to stain, motility, or lack of motility, and often spore formation. Microscopic study may be made either on the living or killed stained bacteria.

The living organisms are most readily studied by the so-called hanging-drop method. A small drop of the material to be examined is placed on a cover glass; this is fastened upon a hollow glass slide so that the drop hangs free from the center. This,

examined under the microscope, will reveal the organisms especially at the edge of the drop; the cells are clearly visible and their motility or non-motility can be readily determined.

Stained preparations are obtained for examination in the following manner: the bacteria are evenly distributed over the surface of a cover glass or glass slide and allowed to dry. These are fixed by quickly passing the slide, or cover glass through the flame, or covering it with special fixing agents. This causes the microörganisms to adhere firmly to the surface of the glass. The surface is then flooded with a solution of one of the aniline dyes, and the glass is allowed to stand a short time, either in the cold or heated; depending upon the nature of the organism being examined. The slide is then washed in water (or, in some cases in alcohol or dilute acid), permitted to dry, and the organism on it examined microscopically. Where it is desired to preserve the specimen, the cover glass is fastened to the slide by means of a drop of Canada balsam.

For further study of the cultures, they are inoculated into various media and their growth characteristics studied. A descriptive chart, prepared and revised from time to time by a committee of the Society of American Bacteriologists, acts as a guide in this work.

Pure cultures of certain pathogens are obtained by inoculating them into susceptible animals. The body of the animal destroys the foreign organisms; whereas, the specific pathogen multiplies in definite organs from which it is later obtained in pure culture.

A few of the very resistant microörganisms may be separated from less resistant species by means of heat or chemicals, but the details of this (as well as the above outlined methods) may be obtained by the student from the many available bacteriology laboratory guides.

CHAPTER IX

CHEMISTRY OF BACTERIA

The child given a new toy proceeds at once to analyze it; for it is an inherent characteristic of the human race to ask of a thing, "Of what is it composed?" In the childhood of the individual, or the race, the answer is often incomplete and speculative, but within recent years man has progressed greatly in analyzing the earth on which he treads; the plants and ani-

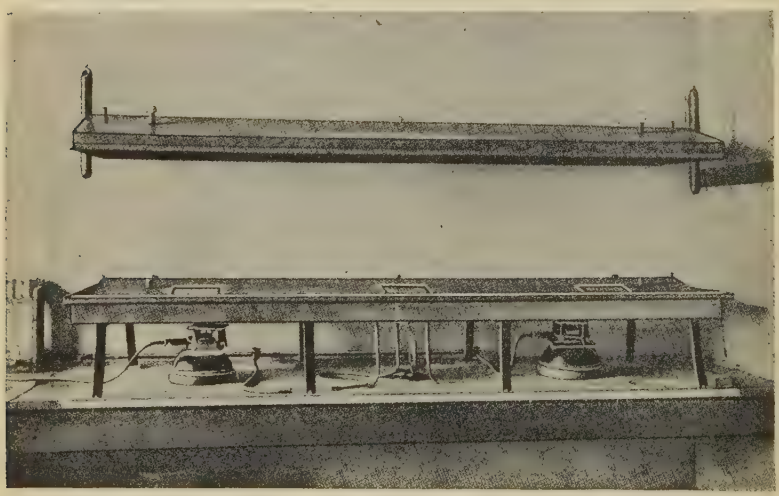


FIG. 36.—Vaughan and Novy's mass culture apparatus. Tanks with raised lid. (*After Vaughan.*)

mals with which he comes in contact; and he has reached out and analyzed the distant stars. Today, with a combination of chemical and microscopic means, he is analyzing not only the bodies of bacteria but also their individual parts.

How Accomplished.—For analysis, the bacteria are grown on laboratory media. Vaughan obtained massive cultures by the use of large copper double tanks (10 feet long, 2 feet wide, and 4 inches deep) around the edge of which were troughs an inch

deep into which were fitted the edges of covers. These tanks were partly filled with a nutrient agar, sterilized, and then inoculated with the desired organisms. The resulting profuse growth was scraped off, washed, and analyzed. The media was resterilized, and in this way several successive crops of the same organism were grown. However, it has been found that they do best when rotated, just as the scientific agriculturalist has learned that he gets the best crops when he practices a system of crop rotation.

The analyst may determine (1) the elements which compose the bacterial cell, (2) the compounds composing it, (3) the composition of the various parts of the cell.

Elementary Composition.—One might expect to find in the body of the powerful little microbe the rare and costly elements, but in this one is disappointed. Bacteria are composed of the common and more abundant elements—the same that compose the bodies of the higher plants and animals. Over 95 per cent of their bodies are made up of the four very common elements: carbon, hydrogen, oxygen, and nitrogen. The other five parts consist of sodium, potassium, calcium, magnesium, iron, sulfur, phosphorus, and occasionally aluminum, silicon, manganese, copper, zinc, and at times still others. Bacteria are more like the animal than the plant in nitrogen content. Wheat contains approximately 2.5 per cent of nitrogen; soy beans, 5 per cent; cow's milk 3.2 per cent; lean beef, 14 per cent; and the bacterial cells as much as 11 per cent. Their phosphorus content is high when compared with that of other plants and animals. This one would expect, when it is recalled that their bodies are composed primarily of nuclear material, and nuclear material is especially rich in phosphorus. The composition of the wheat kernel varies with the variety, the water and the food received during the growing season; likewise, the composition of the bacterial cells varies with the species and the media on which they are grown.

Compounds Composing the Bacterial Body.—The compounds comprising the bacterial body may be considered under the divisions of water and solids. The solids consist of carbohydrates, fats, proteins, ash, and certain less well-defined groups, as vitamins, pigments, toxins, and enzymes.

Water.—Bacteria consist principally of water. The quantity present in the actively growing cell varies from 65 to 98 per cent. The younger the culture, the more active the organism

and the greater the quantity of water present. Old cultures contain considerably less, and when spores form the water content is greatly reduced. However, there is an irreducible minimum below which they cannot go and still maintain life. In fact, the absence of water means the absence of life, and next to living matter water is the most wonderful thing in the world. The ancient philosopher, Thales, considered water as the origin of all things. A little later, Empedocles and Aristotle added to this earth, air, and fire. These were the so-called elements of the ancients. Of these, water, in many respects, is the most interesting; for all that is happening in man, plants, and the lower animals is intimately associated with the transformation of water.

Water is taken up by the roots of the plants; carbon dioxide is absorbed by the leaf, and under the influence of the active rays of light in the presence of chlorophyll there result sugars, starches, cellulose and the whole category of complex compounds found in plants. Each transformation (from a simple to a complex or from a complex to a simple compound) is accompanied by the combination, elimination, or production of water. The complex tissues of plants are taken into the bodies of animals, two-thirds of which is water. Here they are again caused to combine with water (or water is eliminated from them), and there result the mysterious tissues of the specific animal. Or if perchance the animal uses the plant tissues for energy, the end products are principally water, and carbon dioxide. The food in plants and animals is carried into the body by water, and with water the waste products leave.

The temperature of the automobile is kept at working temperature by encasing the cylinders in water and keeping it in rapid circulation. In a like manner, the living organism keeps its temperature normal. The water not only circulates, but it evaporates; hence, its efficiency is increased fifty-fold. Therefore, it is *in* water and *with* water that living tissues are dealing in all their complex processes of life.

Solids.—Solids comprise from 10 to 30 per cent of the bacterial cell. These consist of ash and organic constituents, the latter being composed of proteins, fats. carbohydrates toxins, pigments, and possibly vitamins.

Proteins.—Proteins are complex organic compounds composed of carbon, hydrogen, oxygen, nitrogen and usually sulfur. In addition to these, they sometimes contain other elements,

especially phosphorus. When treated with strong acids or digested by animals or bacteria, they yield certain characteristic compounds called amino-acids. The so-called simple proteins yield only amino-acids, whereas the complex proteins yield amino-acids plus other compounds. Sometimes these are carbohydrates or fats. Others yield amino-acids plus purine bases. These are nucleoproteins. The bulk of the dry matter of the bacterial cell consists of protein. In this respect, the bacteria are more like animals than plants. The proteins which compose the body of various animals differ. Likewise, those composing the body of different bacteria vary. The effects produced in animals suffering with various diseases are due in a measure to these foreign proteins getting into the blood stream, where, according to Vaughan and Novy, they are broken into protein split-products, some of which are toxic.

Another peculiarity of the bacteria is that they consist, to a large extent, of complex nucleoproteins and phosphoproteins. On hydrolysis, these yield the amino-acids and the purine bases, or phosphoric acid.

Lipins.—Bacteria contain numerous organic compounds which are insoluble in water, but soluble in alcohol, ether, and chloroform, and which are greasy to feel. These are known as lipins.

True fats have been recognized in many species by special staining methods. By the use of fat solvents, lecithin, and cholesterol, simple fats and specific bacterial fats have been isolated. The quantity and kind present depends upon the types of bacteria. Approximately 40 per cent of the dry weight of the tubercle bacilli consists of lipins. The amount varies with the strain, the bovine type being richer than the human type. It also varies with the media on which the organisms are grown. The fat-like substance (which the tubercle bacilli carry) probably acts as an armor, protecting the organism against adverse conditions, for these organisms are more resistant than most pathogens. Their peculiar staining properties are due to their high lipin content. Tubercle bacilli are very hard to stain, but when once stained they can be washed in dilute acids without removing the stain. They are, therefore, known as acid-fast bacteria.

Carbohydrates.—Carbohydrates occur in large quantities in all the higher plants, but the majority of bacteria are free from them or carry them only in minute quantities. Some workers have reported the finding of small quantities of cellulose (woody fiber) in some bacteria. However, the cell wall of bacteria consists

mainly of chitin, which, on being digested with acids, yields from 80 to 90 per cent of a nitrogenous carbohydrate, *glucosamine.* This is interesting inasmuch as cellulose is a typical vegetable, while chitin is equally typically animal in origin. The latter is found in the shells of lobsters and crabs and the wings and coverings of flies and beetles. The presence of small quantities of starch, glycogen (animal starch), and some of the sugars, has also been reported.

Ash.—The quantity of ash in the bacterial cell is considerably higher than that in other plants. The same elements are found in both, but the bacteria are peculiar in the large quantity of phosphorus which they contain. At times over one-fifth of the total ash is phosphorus, the remaining four-fifths being composed mainly of potassium, calcium, magnesium, iron, and sulfur.

Composition of Different Parts of the Bacterial Cell.—Having studied the composition of the complete bacterial cell, let us now turn to an examination of the individual parts. Qualitatively the composition of these is determined by the use of various stains, as previously suggested. The outer coating of the cell, the capsule, is a sticky gum-like mass which often causes the bacteria to adhere together. This consists principally of a protein mucin, similar to that found in saliva, or the slimy mass covering the body of snails. Sometimes, as in the case of iron bacteria, they are surrounded by a sheath in which are large quantities of iron. Often, such bacteria are found in water. Water containing these organisms when first drawn from the well appears normal, but on standing the iron in the sheath is oxidized and the water becomes yellow. These organisms may form in large masses and clog the water mains; but from a sanitary viewpoint they are without significance. The cell wall is composed principally of chitin, a compound in composition midway between proteins and carbohydrates.

The inner part of the bacterial cell is composed largely of bacterial proteins. These vary widely with different species; the nucleoproteins and phospho-proteins probably predominate.

Enzymes.—These are the tools with which bacteria do their work. With them they break down the carbohydrates, fats, and proteins. They also use them in the coagulation of milk and in probably most other changes which the microbes bring about. Specific enzymes are used for specific purposes and are properly named from the more complex substance on which they act. This is done by the adding of the suffix "ase" to the root word.

Consequently the enzyme which acts upon proteins are referred to as proteinases. When they occur in the inactive form they are called zymogens. Bacteria use enzymes in the digestion and metabolism of their food, consequently if they have one enzyme they can break down this food; if another, that. So we may say that the enzyme determines the appetite of the microbe. What peculiar appetites microbes sometimes have! Some digest horn, others wood, and still others rock.

A peculiar property of the enzyme is its great activity. A very minute quantity can bring about tremendous changes. A thimbleful of the enzyme which digests proteins could break down a carload of protein, provided it were given the time and no accident happened. If we double the quantity of enzyme we roughly reduce the time by one-half. The reason for this is that each particle of enzyme repeats the same kind of work over and over. It is somewhat similar to a group of men carrying bricks from the ground to the top of a building. Give one man sufficient time and he would be able to transfer the whole pile to the top eventually, but in case many men help in this task, the time required to carry the bricks to the top would decrease as the number of men increased. Occasionally, however, the time used in the work is not directly proportional to the number of men starting. A brick may slip, strike one of the men on the head, and he is through carrying bricks—at least for the time being. A similar phenomenon occurs with the enzymes. Some of them may get entangled in some side issues, and their work is at an end. Moreover, the speed with which an enzyme works depends upon the temperature. Increasing the temperature up to a certain point results in an increase in its activities. Above a certain degree, however, the activity decreases, and if the temperature becomes too high the enzyme is destroyed. Like animals, enzymes are destroyed by poisons. During the life of the bacteria these enzymes are working in harmony, each performing its allotted task—some building up this part of their home, the cell; others, preparing the fuel; and still others, doing away with waste products. Just the minute life ceases, each enzyme commences to work independent of all others, and in time their home (the cell) is torn to pieces. This is likened unto a dance. As long as the music continues, all are in step and there is a certain beautiful rhythm in all. When the music ceases, however, the dancers lose step, bump into each other, and if they continue, all becomes confusion. The enzymes are the dancers in the living

cell. Life is the master musician who furnishes the music. Hence, enzymes manifest themselves in the living and in a degree are subject to the limitations of life; but they are not life.

Pigments.—All have heard of the miracle of the bleeding host. The consecrated bread left overnight in the moist bacterial laden air of the chapel appears on the morrow besprinkled with bright red drops. What could it be? Blood! From whence did it come, and what did it indicate? Various were the interpretations placed upon it and numerous the lives and homes which were sacrificed through this delusion.

The mystery, romance, and tragedy disappear when one sees in the laboratory a tiny organism, *Erythrobacillus prodigiosus*, growing in tubes of starchy food and producing a blood-red pigment. Sometimes, today, bread becomes infected with this organism, and dough which has been set aside overnight not infrequently has been found in the morning to fairly rival the colors of the autumn sunset.

At times on standing, milk develops a very uncanny blue color, which spreads through the dairy like an epidemic. The little culprit which plays this trick is the *Bacillus pyocyaneus*.

In the soil are some nitrogen-fixing bacteria which form a golden-yellow pigment, while others form a chocolate-brown. Some, in water, produce green and various other colored pigments. Thus, we have organisms which produce all the colors of the rainbow.

The phenomenon of pigment production has long attracted the attention of scientists, and many attempts have been made to explain its function; but so far none seem to be wholly satisfactory. The color seems to be of no material advantage to the bacteria, for colorless strains may be cultivated which possess all of the properties of the original strain, with the exception of pigment production. There is no evidence that they protect the organism against light, nor is there anything that would lead one to believe that they are similar to the hemoglobin of the blood, or the chlorophyll of the green plants. The best evidence, therefore, points to the conclusion that they are mere by-products which have no particular value to the organism.

Toxins.—Bacterial poisons are of two classes: first, those resulting from the breaking down of the bacterial body; and secondly, the toxic products actively manufactured by certain bacteria. To the former belong the bacterial proteins which are known as endotoxins. The kind and quantity of this toxin

produced by bacteria vary greatly with different species. Typical examples of bacteria which actually produce toxic substances, or exotoxins, are the diphtheria, tetanus, and botulinus bacilli. The production of the diphtheria toxin is dependent, in a degree, upon the food of the microörganism. This bacterium grown in beef tea produces a highly potent toxin, whereas the same organism grown in the presence of glucose produces lactic acid, the same product found in butter-milk; hence, as pointed out by Kendall, "The story of Dr. Jekyll and Mr. Hyde, that strange and imaginary conception of a dual human personality, has its actual realization (and far more striking and realistic) in the simple experiment upon the energy of the diphtheria bacillus. In plain broth the microbe produces a potent toxin which confers on the bacillus its formidableness in producing disease. The simple addition of energy for the organism so changes the nature of its growth products that it is not only no longer toxic—it is potentially possessed of food value."

Toxins have certain characteristics which differentiate them from other poisons:

1. Toxins are specific, that is, the diphtheria bacterium produces a poison which, if placed in the body of a susceptible animal, will give rise to diphtheria. We can grow the diphtheria bacteria in beef tea and filter off the bacteria; yet the beef tea if given to a susceptible animal will cause the disease, diphtheria. Diphtheria organisms alone produce diphtheria toxin; lockjaw organisms produce lockjaw, etc. Hence, toxins are specific.

2. Toxins are extremely violent poisons, far surpassing any other known poison in this respect.

Smallest quantity of atropin required to kill man....	130 mgms.
Smallest quantity of strychnine required to kill man..	30–40 mgms.
Smallest quantity of cobra venom to kill man.....	4.4 mgms.
Smallest quantity of tetanus toxin required to kill man	0.23 mgms.

The tetanus toxin is produced by the germ which causes lockjaw. The poison has never been obtained pure, yet the crude material is highly poisonous. What a minute quantity of the pure compound would be required to kill a man! Experiments show that only a few molecules of the botulinus poison, a poison sometimes found in food, is required to kill small animals.

3. Bacterial toxin if used in extremely small but increasing doses will produce immunity. When a very minute trace of the toxin gets into the body of an animal, the cells begin to manu-

facture an emergency material which robs the toxin of its power. This is known as antitoxin, and the presence of it in the blood of the individual means that he will not take lockjaw, diphtheria, or whatever the specific disease. We then say he is immune. The immunity may become so great that it makes no difference how many of these poisonous darts are shot at the individual by the microbe—they fall without piercing his armor of immunity. However, it is different with strychnine. To this, the individual never becomes resistant. Man may become able to tolerate more nicotine, but give even the habitual tobacco user a large dose of nicotine and death will result.

4. Toxins have an incubation period, whereas most ordinary poisons have not. If an animal receives strychnine it soon becomes ill and quickly recovers, or else dies; whereas if an animal is given a bacterial toxin there may be a delay of days or even weeks before the symptoms appear. The time between the entrance of a toxin into the body and the appearance of symptoms is known as the incubation period. It varies with different toxins and also with the quantity of toxin administered.

5. Toxins are easily destroyed by drying, sunlight, heat, and certain chemicals. Thus, any material containing bacterial toxin, if heated to 100° C. for fifteen minutes, will be free from it. However, if strychnine is treated in this manner, it does not lose any of its powers. A toxin may be heated and, thus, lose its power of poisoning an animal, but still retain its power of calling out "anti-toxin," thus rendering the animal immune to the disease. This is used very extensively in medicine to protect us against disease.

Ptomaines.—These are products produced when some bacteria decompose proteins under anaërobic conditions. Some of them are poisonous; many are not. It was once thought that most food-poisoning was due to ptomaines, but it is now known that they are seldom, if ever, the cause. They have none of the properties mentioned above which are possessed by toxins.

Light.—Most individuals have been mystified and delighted, if not frightened by the uncanny, beautiful light which sometimes covers a stump in the forest. At times, especially along the seashore, it seems to be emitted as a mysterious yet beautiful phosphorescence from the sand. One writer tells of a cluster of sausages brought to his laboratory. It was hung up in a dark cellar and when the maid went for it in the morning there "hung in the place of the sausage a fiery effigy which seemed to her more

like the quondam spirits of their mysterious ingredients than the unctuous homely friend of the homeless boarder."

The explanation of these apparent mysteries is that certain bacteria live on decaying wood, leaves, salt water, meat, and fish, and emit a light.

The production of light depends on the temperature and food supply. The phenomenon has been seen at temperatures as low as $-20°$ and as high as 45° C. The luminous bacteria seem to like salt and to require oxygen, for when the latter is exhausted in their media, the luminosity ceases. If a bubble of air is made to pass up the side of a tube containing a culture of luminous bacteria, which has just ceased to emit light for lack of oxygen, a wave of light may be seen to pass along the tube. However, the actual quantity of free oxygen required is very small. In some cases, one part of oxygen in about 143,000 parts hydrogen by volume was found sufficient. This corresponds to one part oxygen by weight dissolved in 3,700,000,000 cc. of sea water.

Strains of organisms have been produced by natural selection so luminous that their light could be seen in full daylight. It has been possible to obtain sufficient light from some of them to permit the reading of time from a watch or moderately large print in dark rooms. It has been suggested that they be employed as lamps in powder magazines or mines, for they emit no heat. Certain fishermen employ, as bait, luminous fragments of dead fish, made so by bacteria.

Heat.—Probably all bacteria liberate heat; there are a number which liberate it in sufficient quantity to change the temperature of the medium in which they grow. This is observed in the heating of fermenting silage, manure, and hay. At times, the temperature is raised to the kindling point, with the result that spontaneous combustion may occur in hay and grain stacks. Bacteria generate considerable of the heat, but other chemical processes are also active. This is made use of in the building of cold frames. A heavy dressing of fresh horse manure is placed under the top soil. This decomposes and furnishes sufficient heat to force the plants.

CHAPTER X

FOOD REQUIREMENTS OF BACTERIA

All living protoplasm requires food. Some forms require it only for growth and repair, whereas others need it also as a source of energy. The energy of sunlight is locked up in chemical compounds by green plants. These, when used as food, are decomposed and built into specific protoplasm, or the energy is liberated for use in life activity. Bacteria are chlorophyll-free plants; hence, they require food for growth, repair and energy.

Food.—The term, food, in its broadest sense, may be considered as including everything taken into the body which is essential to the life of the body and its activity. In this sense, carbon dioxide, water, and the mineral elements are often spoken of as the food of plants. They are useful only to the green plant and to these only in the presence of light. To chlorophyll-free plants (or to plants in the dark, and to all animals) they are not food. For this reason, it is stated that green plants build their own food from the simple inorganic constituents—water, carbon dioxide, and the minerals of the soil. Thus are produced storehouses of potential energy which are used by other organisms not so favorably constituted.

Bacteria are unable to use the kinetic energy of the sunlight in the production of their food, but some of them can utilize that of inorganic compounds, and in the presence of the required elements build their own food. However, the majority of bacteria obtain their food, ready formed, from other plants or animals; hence, from the viewpoint of bacteriology, we may consider food as any substance which bacteria can utilize in obtaining either building material or energy for cell activity. It may be that in the future this definition will be enlarged to include substances which are taken into the body and govern cell activity, as is the case with the animal cells.

Although the forms of food required by different bacteria vary greatly, they must all contain ten of the known elements. These, all of them very common, are: carbon (C), hydrogen (H), oxygen

(O), nitrogen (N), calcium (Ca), potassium (K), magnesium (Mg), sulfur (S), phosphorus (P), and iron (Fe). Using the key for remembering, as suggested by that great teacher, Cyril G. Hopkins, we have: C. Hopk'ns Ca, Fe, Mg—"C. Hopk'ns Cafe—Mighty good."

Food may be divided into three great classes: organic, ash, and water. The organic compounds comprise the four great groups: carbohydrates, fats, proteins, and vitamins. The first two are composed of carbon, hydrogen, and oxygen; whereas, the proteins contain in addition, nitrogen and sulfur, and sometimes other elements.

Quantity of Food Required.—Bacteria are like animals in that the quantity of food needed and that actually used in the presence of an abundant supply is widely different. Some bacteria will actually multiply in distilled water. In such water, they may multiply until there are billions in a gallon, obtaining the food and energy for their life processes from the minute traces of impurities present. Other bacteria will grow on the surface of granite, obtaining their required food from the small quantity of foreign matter brought to them in the rain water.

On the other hand, the maximum quantity of food used by bacteria may be enormous. They quickly decompose the bodies of dead plants and animals. Tons of material are carried into the septic tanks of large cities, all of which are rapidly used by bacteria.

The food required by each bacterial cell is not great, for it would take 1,600,000,000 colon bacilli to weigh approximately one milligram. The waste products and repair material would make the cellular requirements slightly greater than this, but the figures are sufficiently accurate to indicate that the quantity needed for building material for one cell is exceedingly small. Their great need for building material is brought about by the rapidity of their multiplication. Colon bacilli placed in favorable environments may divide into two daughter cells every fifteen minutes. The theoretical 24-hour progeny of a single colon bacilli therefore would be 2^{96}, or nearly 8×10^{28}, a truly enormous number—a number which would have a very appreciable mass. After the first few hours natural barriers (such as the lack of food, accumulation of waste products, and mutual antagonism) retard the multiplication of bacteria. Nevertheless, the rapid multiplication of bacteria is one of the factors which make their food requirements so great.

As pointed out by Kendall, another noteworthy characteristic of the bacterial cell is its disproportionately large ratio of surface area as compared to weight or volume. Thus, the typhoid bacillus which is a microbe of average size, has a volume of approximately 0.000,000,002 cc. and a weight of about 0.000,000,002 mgm. but a surface of 0.000,01 sq. mm. In no other living organisms (except possibly the filterable viruses) is this ratio of surface to volume equalled. The energy requirements of living things in general are determined to a great extent by the ratio of their surface to their volume. Hence, the energy requirements of bacteria are great. Moreover, many only partly digest their food, leaving most of the energy in the by-products. For instance, microörganisms, which completely oxidize sugar to carbon dioxide and water, will obtain thirty times as much energy from a given quantity of grape sugar as will others which change it only to alcohol.

How Bacteria Feed.—Animals take food into their stomachs and secrete digestive juices, which are mixed with it and render it soluble and assimilable. It is then taken up by the blood, carried to the different parts of the body, and there used to build new tissues, to repair old worn out ones, or to furnish energy to the animal. Bacteria have no stomachs; consequently, they pour their digestive juices directly into the medium in which they are living. After digestion, the food is absorbed. This very fact makes the bacteria of economic importance, as many of the digestion products are of value to man; moreover, some of the food of plants are digestion products of the bacteria.

Kinds of Foods Required.—Bacteria feed upon a great variety of substances. The sulfur, iron, and nitrifying bacteria are able to live upon mineral nutrients. The sulfur organisms obtain their energy through the oxidizing of sulfur dioxide to sulfuric acid. The nitrifiers oxidize ammonia to nitrites and then to nitrates. Both classes obtain their building material from simple inorganic compounds; consequently, they can live without plant or animal tissue, in the absence of sunlight. It is this function that leads us to believe that they probably were the first inhabitants of this globe. The majority of bacteria cannot live upon inorganic compounds, but like animals require more complex foods. The ordinary decay bacteria thrive on any plant and animal tissue. Woody fiber, bone, horn, and proteins of all kinds are rather rapidly consumed by them. Other organisms, as for instance those causing tuberculosis, are more particular

in their food requirements, growing best on blood and tissue extracts. Others, as for instance the leprosy organisms, appear to grow only in living tissue.

Those bacteria which require neither organic carbon nor organic nitrogen, but live upon minerals, as do the higher plants are often referred to as *autotrophic* bacteria; those which feed upon dead plants and animals are called *heterotrophic* or *saprophytes;* and those which feed upon living plants and animals are referred to as *parasites.* The first two classes contain the beneficial soil organisms, whereas the parasites are the disease producers. This latter group is often spoken of as the pathogens. A microörganism may live at one time as a parasite, and at another as a saprophyte. Some few bacteria appear to live only as parasites. In time, we may learn how to grow all so called obligate parasites on artificial media.

Water.—Man can live for days without food, but only for a few hours without water. The same is true of bacteria. Remove all the water from the medium in which bacteria are growing and they soon die or pass into the spore stage. The pneumococcus, the cholera spirillum, and the Pfeiffer bacillus die in a few hours on drying; whereas the typhoid, diphtheria, and tuberculosis organisms may survive for days. The tetanus, anthrax, and many soil organisms resist drying for months or even years. Of course, in the case of these resistant organisms the water is never completely removed; otherwise, they would quickly die.

Water plays a number of parts in bacterial life: (1) It dissolves the food so it can be taken up by the bacteria. When the food is not already soluble the microbe throws off an enzyme which brings the food into solution so it may be taken up by the cell. (2) Water carries the waste products from the cell. (3) It enters into nearly all the changes going on within the cell. When starch is being digested, water enters and the starch breaks into sugar. Fats break into glycerine and an acid through the taking-up of water. In the digestion of proteins, every step is accompanied by the taking-up of water. When bacteria are building-up in place of breaking-down, water is the pivot around which all reactions turn. When food burns within the body or within the stove, water is continually being evolved. (4) Water is the agent that speeds up all reactions. If a match becomes wet, it will not ignite; nor will it if the air, match and surface on which it is rubbed is absolutely dry.

It is the same with the changes going on within the cell. Water must be present or the cell reactions will not proceed normally. (5) Water enters largely into the composition of the cell, as bacteria consist of from 70 to 90 per cent water. (6) Water gives to the cell its shape. If we place a cell in a strong salt solution, water is drawn from it. If we place it in distilled water, it bursts. The force which causes the bursting is called osmotic pressure. The extent of this pressure varies with different cells. It averages about the same as that of a 0.9 per cent salt solution. This is about 7.1 atmospheres. In such a solution, the tissues neither gain nor lose weight.

Carbon Requirements.—All living protoplasm require carbon. But, how varied are the forms in which it is taken! Green plants take it up as carbon dioxide; man, as carbohydrates, fats and proteins. Probably all forms of carbon, except the diamond, are utilized by some bacteria. Some soil organisms can utilize the carbon of carbon dioxide; others, that of formaldehyde; while still others can utilize the carbon of carbolic acid. Both the soil and manure contain bacteria which use the carbon of urea; from some of it they construct their bodies, but most of it is changed into ammonia which is used by other microbes. The woody fiber of plants yields its carbon to bacteria, and there are writers who claim that coal is produced by the action of bacteria upon plants. Renalt, for example, describes fossil bacteria which he found in coal, and today we know microbes which get their required carbon from coal, and also bacteria that produce the coal-like material occurring in soil. Most bacteria, however, prefer their carbon in the form of carbohydrates. Many pathogens growing in the presence of them, produce non-toxic acids, but if grown in the absence of carbohydrates produce very potent toxins. Bacteria are very fastidious in their carbohydrate requirements. Some bacteria will pick all of one sugar from a medium and leave untouched a nearly related compound. This characteristic is often made use of in identifying or separating nearly related compounds. Such reactions are far more delicate than those made by the most refined chemical reagents. For instance, available information indicates that as small an amount as 0.0025, or possible 0.001 per cent, of glucose in an appropriate cultural medium may be detected by the use of suitable bacteria. This method can also be used to identify bacteria by seeding the unknown organisms into the various sugars and noting the one which it digests.

Proteins are split by some bacteria, and the carbon of the resulting amino-acids utilized for energy or building material; however, many bacteria cannot split the protein molecule and

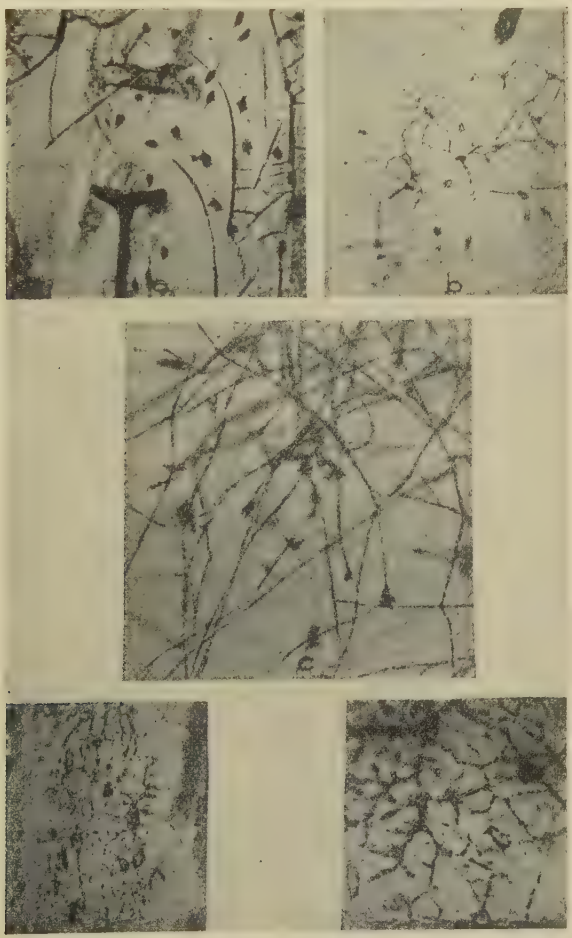


FIG. 37.—Carboniferous bacteria and fungi as figured by Renalt. (*After Moodie.*)

only utilize the carbon of proteins after they have been broken into amino-acids by animals or other bacteria.

The carbon in the fat molecule is more resistant to the attack of bacteria For this reason, it often gives considerable trouble to

the plumber. Fatty materials are thrown into the sink; they find their way into the sewer; bacteria dissolve the accompanying substances and the fat is liberated. This collects around some object, as for instance, a match. As this is rolled along by the water it gathers more fat, until at times, it becomes large enough to clog a pipe. Proteins yield their carbon readily and often in the breaking down of them, as for example in the egg, the sulfur is changed to the ill-smelling gas, hydrogen sulfide. A few of the pathogens require their carbon as the complex body tissues and fluids, and a very few at the present time seem to require that this tissue or fluid be in the body of a living animal.

Some of the carbon is used by the microbe in building cell protoplasm. However, most of it is used as a source of energy. Inasmuch as the energy requirements of microörganisms are relatively great, carbon is required by them in larger quantities than is any other element.

Nitrogen.—Nitrogen in the free form is one of the inert elements. However, when once cajoled into joining hands with carbon and oxygen, we obtain wonderful compounds. The most beautiful dyes contain nitrogen. It is tucked away in the most powerful explosives, ready on the least provocation to become excited and blow everything to pieces. The majority of delicate perfumes and powerful poisons contain it. But most wonderful of all, it is the basis of living protoplasm! All microörganisms require it in one form or another. The nitrogen-gathering organisms of the soil can cause the inert atmospheric nitrogen to join hands with carbon, hydrogen, and oxygen and thus produce their mysterious bodies. There are bacteria in manure which transform urea into ammonia. Others oxidize the ammonia to nitrites, and still others, in obtaining their energy, transform the nitrites into nitrates. Many bacteria can use the nitrogen in egg, milk, flour, and all kinds of plant and animal tissue; others require more complex nitrogen compounds, as for instance such as are found in living plant, and animal tissue. Sometimes the protein compounds are pulled to pieces with the formation of ptomaines; at other times, they are built into those most powerful of all poisons, toxins. Much of the nitrogen, however, is used in building that wonderful little engine, the body of the microbe. Microörganisms break down the complex proteins into simple building blocks before they are built over into their bodies just as man would reduce the wall of an old church before building it over into a new schoolhouse.

Soil bacteriology deals with the soil organisms and to a great extent their relationship to the nitrogen supply. Many bacteria are busy changing the nitrogen compounds into forms which are acceptable to the higher plants.

Oxygen.—Oxygen is essential to all forms of life. Some bacteria require it free, others can use it only when combined. To those which can live only in free oxygen, Pasteur gave the name, *aërobes* and to those which live only in the absence of free oxygen, *anaërobes*. Some can become adapted to the use of combined oxygen. These are known as *facultative anaërobes*. Free oxygen is a poison to absolute anaërobes. True aërobes are great lovers of oxygen; and if shut up within a little cell containing a few drops of water in which is enclosed a bubble of air, on examination under the microscope it will be seen that the motile ones will make their way from all parts of this tiny ocean and cluster around the drop of air.

Hydrogen.—Hydrogen is required by all microörganisms. There are some which can use hydrogen gas, but most of them obtain it in combinations such as water, or the various organic compounds.

Ash Requirements.—Animals fed on a diet of pure water, ash-free proteins, carbohydrates, and fats die sooner than if given only water. Likewise, bacteria require mineral nutrients. They use them for various purposes: (1) For maintaining the osmotic pressure of the cell. These give to it its plump contour and assist in the taking up of foods. (2) For the neutralizing of acids. The nitrifying bacteria and many other microörganisms produce acids, which, if permitted to accumulate, would soon cause the death of the cell. (3) Some of the minerals are built into the body of the microbe and give to it its peculiar chemical and physical properties. Some bacteria use sulfur for energy. These are comparatively large organisms and have when taken directly from sulfur springs yellow granules of sulfur within their bodies. On fasting the bacteria, these granules soon disappear, and sulfur dioxide, hydrogen sulfide, or sulfuric acid appear in their place, depending on the specific microörganism in question.

Vitamins.—Vitamins are essential to the well-being of all animals. They are produced by green plants, and there is evidence today that bacteria can also synthesize them. Although they may not be essential to bacterial growth, there are, nevertheless certain accessory food substances which exert a favorable influence on bacterial development. Davis and Ferry describe

a remarkable stimulating influence on the growth and toxin production of the diphtheria bacilli by the addition of small quantities of vitamin-containing substances to the medium in which the bacteria were growing. Several other workers have likewise noted a somewhat similar stimulation in the growth and development of the azotobacter and streptococci.

Mutual Relationship between Plants.—There are some species of microörganisms which live in close association with other plants and in this manner get their food. The legume bacteria live in the small nodules of the alfalfa plant, drawing from it the food needed, and giving the alfalfa nitrogen compounds in return. Such a friendly association plays a prominent part in soil bacteriology, for probably, there are many microörganisms which thus associate with one another.

Sometimes, one microbe changes the food so that another can readily use it. There are bacteria in the soil which break down plant tissues into acids which are used by the nitrogen-fixing bacteria. The yeast acts on sugar and changes it to alcohol. This can be used by bacteria in producing vinegar. Such an association is known as *metabiosis.*

There are other species of bacteria which are mortal enemies and cannot live together, one species killing out the other almost as soon as they come in contact with each other. The details of this minature warfare are not well understood, nor do we, in all cases, know the weapon by which it is carried on. It is probable that some poison is secreted which is without effect upon its producer, but deadly to its antagonist. This is well illustrated by the following example. Meat if left by itself soon spoils, but if dropped into buttermilk will keep some time. The lactic acid formed by the milk organisms is a deadly poison to the putrefying bacteria. This antagonistic action is called *antibiosis.*

CHAPTER XI

PRODUCTS OF BACTERIAL ACTIVITY

Bacteria are of economic importance for three reasons: (1) Many act as scavengers, clearing away the dead and useless bodies of plants and animals. (2) Some bacteria produce products which are valuable to man. A number of these substances are used directly. Some favorably influence man's food, while still others are food for the growing plants. (3) A few bacteria produce compounds which are poisonous to man and the lower animals.

Metabolism.—Living organisms are peculiar in that they can take materials very unlike their own bodies and build from them their specific tissues. They can also liberate the energy contained within organic compounds and use it in their body activities. This necessitates the changing of the substances. Sometimes it is a building of more complex from simpler compounds: *anabolism*, or *anabolic* processes. More often it is a breaking up of complex compounds into simpler substances; *catabolism*, or *catabolic* processes. The total transformations going on in the plant, or animal body are known as *metabolism*, a term which really means "transformation of matter."

The metabolism of bacteria has commanded the attention of scientists of recent years not only because of the economic importance of the resulting products, but also because it opens the way to a better understanding of the transformations going on in the human body. Bacteria use the same foods as man. From them they build complex compounds, and what is more important, they oxidize them. Man is able to obtain these oxidation products from the bacteria long before these products have reached the final stage of carbon dioxide and water. From a study of these fragments scientists are formulating theories as to the way in which the cell works in obtaining its energy. This has resulted in working theories which can be applied to the animal organism.

Chemical Products.—Jordan divides the products produced in bacterial life into four classes: (1) secretions, (2) excretions,

(3) disintegration products of bacterial activity, and (4) true cell substances. The secretions, those substances which serve some useful purpose in the cell activity, may be retained within the cell or pass into the surrounding medium. Enzymes are typical examples of these products and have been considered in a previous chapter. The excretions, those substances which have been ejected as useless to the cell, are the ashes of cell metabolism. Nitrous, nitric, and carbonic acid are examples. The disintegration products of bacterial activity consist of the various organic acids, nitrogenous constituents, and ptomaines. The true-cell-substance group includes the protoplasm and the products which are transformed into protoplasm.

Fermentation.—The term fermentation, as used in bacteriology, applies to the biochemical decomposition of nitrogen-free compounds, chiefly carbohydrates, caused by the action of microörganisms. Sugars, starches, celluloses and their derivatives are broken down in this process with the formation of alcohols, acids and other substances, and finally carbon dioxide and water. It differs from putrefaction in that the products usually are not ill-smelling and comparatively non-toxic.

Alcohol.—Alcoholic fermentation is due principally to yeasts. Some molds and a very few bacteria also produce it. When alcohol is formed by bacteria it is produced only in small quantities and always accompanied by several acids. In fact, as produced by all microörganisms it is associated with other products: glycerine, fusel oil, succinic, acetic, and lactic acid. The quantity and kind of each product depends upon the material being fermented and the specific microörganism bringing about the change.

The classic work of Pasteur on the so-called "diseases of wine," led to a definite knowledge of the processes involved in the making of alcoholic liquors. In the alcoholic fermentation of fruit juices, sugar is the substance decomposed. In bread dough and in beer-wort it is malt sugar (maltose derived from the flour of the germinating grain). The same is true of corn and potato mash (from which Bourbon and Irish whiskies are obtained). The variation in wines produced in different districts is due to the specific microörganisms which find their way into the fermenting mixture. There are strains in some districts superior to those found in others just as superior strains of flowers and plants occur in various localities. However, the modern manufacturer does not depend upon chance-infection, but grows within his own

laboratory the superior strains which are used to inoculate the substances to be fermented. Various substances may be used in the manufacture of alcohol. In this country the chief substance is sugar-cane molasses; in Germany, potatoes; in France, sugar-beets; and in many other countries, sorghum is used.

The sugar-cane molasses is diluted with water and a little acid in order to give the yeast better working conditions. A pure culture of yeast is added and fermentation permitted to continue for about three days. The carbon dioxide given off is collected, purified, and stored in cylinders to be sold for use at soda fountains. The resulting mash contains from 6 to 8 per cent alcohol, which is pumped into a still and distilled. This crude distillate contains many impurities—aldehydes, esters, and higher alcohols. These are separated by rectification. By this means, alcohol of a very high state of purity is obtained.

In the first stage of vinegar production, alcohol is produced by yeast, which is later oxidized to acetic acid by bacteria.

The various changes which occur in dough that is made from flour, and before it is baked, are known as panary fermentation. The product formed consists essentially of gas, usually as a result of alcoholic fermentation brought about by yeast. However, there are other products, especially acids, the nature of which depends upon the leaven used in making the dough. During the leavening process, the gas is produced which causes the porous light condition of the resulting bread. Products which contribute to the flavor are also formed. The baking destroys most of the microörganisms and drives off much of the alcohol, but the lactic acid, which has been produced, remains.

In parts of Europe and Asia preparations containing alcohol are produced by the fermentation of milk. This occurs most readily in milk containing relatively large proportions of milk sugar (such as sheep's, mare's, and camel's milk). The three principal forms will be briefly considered here.

Leben.—This was produced by the ancient Egyptians, Arabs, and Carthaginians from the milk of buffaloes, cows, and goats. It is usually prepared by boiling the fresh milk over a slow fire, after which some fermented milk from a previous preparation is added to the warm article. The fermentation takes place rapidly and is considered to be complete at the end of six hours. The product is valued highly and is offered in hospitality to the passing stranger. In fact, in some parts of Arabia payment for this delicacy would be considered an insult.

The fermentation is due to the action of yeast and bacteria which produce both alcohol and acids. Some of the half-civilized tribes of Siberia prepare a strong alcoholic beverage by the distillation of leben. The distillate contains volatile acids, and from 7 to 8 per cent alcohol.

Koumiss.—This is the most famous of all fermented milks. It has been known from the most ancient times to the present day as the principal food of the wandering tribes who inhabit the steppes of European Russia and south, western, and central Asia.

It is prepared by mixing one part of warm water with five parts of mare's milk. To this is added some old koumiss, and the mixture agitated until fermentation occurs.

The change undergone during fermentation consists of a vigorous production of gas and alcohol, accompanied by an acid formation which causes the coagulation of the milk. The coagulum exists in an extremely fine state of division, and the liquid froths violently when the bottle is opened. It is reported to have a full, pleasant acid taste and contains from 1 to 2 per cent alcohol. The fermentation is due to the combined action of bacteria and yeast. The comparative absence of pulmonary tuberculosis among the tribes which use this product in some cases has led to its exploitation as a cure for consumption.

Kefir.—Kefir is a kind of fermented milk which has long been used in the Caucasus. It differs from koumiss in that it is prepared from either sheep's, goats', or cows' milk. The fermentation is started by the addition of a few grains of kefir to the milk (which is contained in leather bottles). The kefir grains are small solid kernels which are kept in families and handed on from one generation to another. Traditions are prevalent among the Mohammedan tribes of the Caucasus that kefir grains were in the first instance presented by Allah, to a preferred tribe as a sign of immortality. Others hold that, in past ages, they were found by shepherds growing on a shrub in the Caucasian highlands; whereas, according to Skolotowski, they were originally found adhering to the walls of oaken vessels used in the preparation of Airam, a sour milk beverage similar to kefir. They are a zoögloea composed of bacilli and yeasts.

Other microörganisms are known which produce the more complex alcohols—butyl and amyl alcohol. The latter is fusel oil and often causes poisoning when crude alcoholic fermentation products are consumed.

Acid Production.—Many microörganisms decompose carbohydrates, sugars, starches, and cellulose with the production of acids. Ever since Pasteur showed that the production of acid is brought about by the action of microörganisms, considerable attention has been given to this subject. This is due to the fact that (1) many of the acids produced are of commercial importance, and (2) the property of microörganisms to produce various acids under definite conditions is so specific that the process may be used to identify various organisms.

The quantity and nature of the resulting acid depends upon the species of microörganisms used and the media in which they are grown. Some organisms, as for instance the *aciduric* (acid-loving bacteria), not only produce and survive in the presence of considerable quantities of acid, but their proliferation is apparently dependent upon the ultilization of carbohydrates or carbohydrate-like substances. Prominent members of this group are *L. acidophilus* and *L. bulgaricus*.

There are other organisms which will grow either in a carbohydrate or carbohydrate-free media, but the production of acid occurs only in the presence of a carbohydrate. The following are some interesting examples of this phenomenon: The diphtheria bacillus does not form its potent, soluble toxin in a glucose-protein medium, but produces lactic acid. In the absence of glucose the Shiga bacterium fails to manufacture its characteristic poison. It also produces lactic acid. Indol is not produced by the colon bacillus in the absence of a carbohydrate, nor does the *Bacillus proteus*, produce its proteolytic enzyme under such conditions. Both produce acid products, mainly lactic acid. The organisms, in the presence of carbohydrates, get their energy from them; hence, carbohydrates become sparers of proteins, just as they do in the animal and human body.

The more important acids produced by bacteria are: acetic. lactic, butyric, oxalic, propionic, hippuric, succinic and formic. Only the first three will be given brief consideration.

Acetic Acid.—It has been known for a long time that when beer, wine, and similar alcoholic liquors are left exposed to the air a tough, mucilaginous film or skin develops on their surfaces; the alcohol gradually disappears, and vinegar results. It has also been known that a small portion of this film, if transferred to non-fermenting liquors, causes them to ferment; hence, the substance has become known as mother of vinegar. Some shrewd guesses were made that the mother of vinegar was com-

posed of living entities, but even as late as the last half of the nineteenth century, this was not the generally accepted idea. In 1864, that illustrious Frenchman, Louis Pasteur, proved that this fermentation is a physiological process, the inception and maintenance of which is bound up with the vital activity of micro-örganisms to which he applied the name *Mycoderma aceti.*

Since that time many species of aërobic acetic-acid bacteria have been described. All of them are bacilli that usually grow in chains and do not produce spores. Some produce only small quantities of acid; hence, they have no commercial value. Others produce large quantities and are used in vinegar-making.

The acid results from the oxidation of the alcohol by an intracellular enzyme. This action proceeds best at a temperature of 20° to 30° C. in the presence of an abundance of oxygen and in a medium containing suitable nutrients. The greatest commercial value of these organisms is in the manufacture of vinegar.

Butyric Acid.—Fermentations which result in the production of butyric acid, are common. Before Pasteur reported his work on acid fermentation, this condition was studied and reported by him as due to the action of bacteria. The organism causing butyric fermentation is now recognized as a bacillus, but Pasteur considered it to be an animal, for he found it actively motile. However, the phenomenon which especially attracted his attention was its ability to live without air. The organisms are anaërobes. Butyric fermentation is of importance chiefly because it imparts to food in which it has occurred a bad odor and flavor. Ordinarily, butyric acid bacteria are inhibited from developing in food by the acetic or lactic acid present. However, the producers of these latter acids are easily destroyed by heat, whereas the butyric-acid bacteria form spores which may survive steaming for an hour and a half. They occur in the soil, and when they find their way into food products are hard to destroy. They sometimes cause the spoilage of canned goods; such as peas and beans.

Lactic Acid.—When milk is allowed to stand, it develops a sour taste and curdles. Investigations show that this flavor is due to lactic acid which is produced from a portion of the sugar, lactose, already present in the milk. The curdling comes from the precipitation of the milk casein by the lactic acid. This apparently spontaneous fermentation is the result of the vital activities of the bacteria which gain access to the milk after it is

drawn from the cow; for if special precautions are taken to prevent their admittance or to destroy them by heat or other means after they have entered, the milk may be kept unchanged for an indefinite period.

Pasteur was the first to describe an organism characteristic of lactic fermentation and to prove the same capable of producing acid in sweet, sterile milk. He named it the "ferment of lactic fermentation," and since that time the bacteria have been obtained and used in pure cultures. Many different species of bacteria are capable of producing lactic acid in milk, yet the principal ones responsible for the natural souring of milk belong to two groups—*Streptococcus lacticus* and *Lactobacillus.*

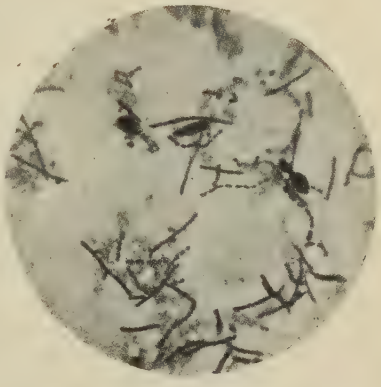


FIG. 38.—Microphotograph of preparation from Armenian soured milk. (*After Douglas.*)

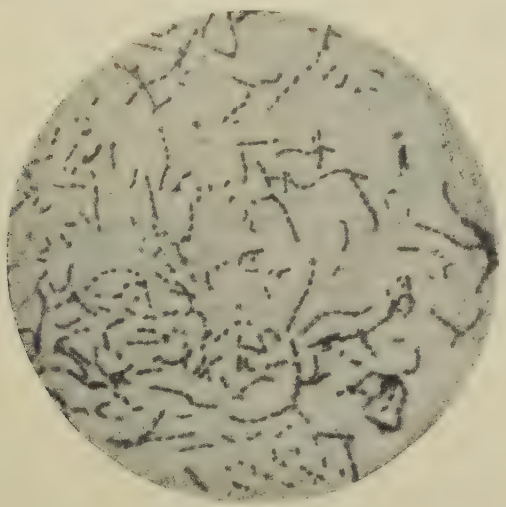


FIG. 39.—Streptococcus lacticus. (*After Douglass.*)

To the producer and vender of fresh milk these organisms are objectionable, for at the proper temperature (30° to 35° C.), they

quickly sour the milk. Hence, precautions are taken to prevent the heavy seeding of the milk with these organisms, and it is kept cold to prevent their rapid multiplication. In the production of butter the lactic acid bacteria play an important part in the ripening process to which cream is subjected before churning. At times, this fermentation is allowed to occur spontaneously depending upon the organisms which by chance find their way into the milk; at other times, buttermilk or sour milk is added to hasten the process. However, in the best factories today pure cultures of the lactic acid bacteria are used as starters. This latter process insures a product of more uniform composition.

Putrefaction.—Proteins are highly complex compounds composed of carbon, hydrogen, oxygen, nitrogen and sulfur. For the most part they belong to that group of compounds referred to as colloids. On digestion they are broken into specific products, amino-acids, some 18 or 20 of which are known. Bacteria, break down proteins, and thus produce from them these amino-acids. However, they go a step farther and disrupt the amino-acids with the production of ill-smelling compounds. Hence, putrefaction is literally a rotting or offensive decay of proteins, and, as Pasteur first pointed out, is essentially an anaërobic process brought about by various bacteria. The end products of putrefaction are ammonia, nitrates, carbon dioxide, hydrogen-sulfide, and the like—all simple, stable non-poisonous compounds in the ordinary concentration. However, the intermediate products resulting from the partial decomposition of proteins may be toxic. The quantity and nature of these products depend upon the specific bacteria and the media in which they are growing. In this group are the so-called ptomaines which were referred to in an earlier chapter. They have been defined by Vaughan as organic chemical compounds, basic in character, and formed by the action of bacteria on nitrogenous matter. Most of them are non-poisonous. Two-thirds of them contain only carbon, hydrogen, and nitrogen. The others also contain oxygen. These latter are the more poisonous. Some members of this group are: sepsine, cadaverine, putresine, choline, muscarine, and neurine. The last two, which are quite similar in action, are very poisonous, and have been obtained from poisonous mushrooms. It is possible that they also play a part in cattle-poisoning which sometimes results from the feeding of fermented beet by-products.

Alkali Production.—In sugar-free media many bacteria produce an alkali. Among the principal ones formed is ammonia, which plays a leading rôle in the bacteriological changes occurring in soil. It is first produced as ammonia and later changed into nitrates. The production of alkali depends upon the microörganisms bringing about the change and also the medium in which these microörganisms grow. There may be the production of an acid followed by that of an alkali. For example, milk when allowed to stand increases in acidity up to a certain concentration, after which the acid disappears. Later the milk becomes alkaline due to the production of ammonia by the breaking down of the casein.

CHAPTER XII

INFLUENCE OF ENVIRONMENT UPON BACTERIA

All living organisms are dependent upon certain environmental factors. Change the environment, and the organisms either perish or adjust themselves to the new conditions depending upon the specific organism, the magnitude, and the nature of the change. Microörganisms are unique in that many of them withstand great environmental variations and apparently adapt themselves more quickly to new conditions than do the higher plants and animals.

Temperature.—The probable temperature limit at which bacterial life is possible is absolute zero (−273° C.) and 160° C. The temperature at which they can grow and carry on their other life activities is much narrower (0° and 90° C.). The rapidity with which they grow and function varies with the temperature. This is a universal law holding throughout the plant and animal kingdom. Moreover, the rate of variation due to temperature is of the same magnitude as that occurring in chemical reactions in general. This principle is illustrated by experiments of Loeb and Northrop on the fruit-fly. They hatched flies under aseptic conditions and fed them on sterile food in containers free from microörganisms. These flies were grown at temperatures varying from 10° to 30° C. Those grown at 30° C. (which is not far from the temperature at which this species of flies normally live) had an average life of 21 days. At 20° C. they lived 54.3 days, or a little over twice as long. At 25° C. they lived 38.5 days; and at 15° C. 123.3 days, or about three times as long. These investigators, showed that the length of the fly's life is inversely proportional to the temperature at which the fly is kept, and that for each 10° drop in temperature the life of the fly is doubled or trebled. The flies kept at the lower temperatures moved about but were very sluggish; (they lived slowly but for a long period). Those kept at the higher temperatures were very active. They lived fast and only for a short time.

Bacteria at a low temperature multiply slowly and produce only small changes, but at higher temperatures their life activi-

ties are rapid. This is well illustrated in the case of meat. In cold storage it may be preserved for a year; in the warm kitchen it spoils in a few hours. In the first case, bacterial activity is slow; in the second, rapid.

There is for every microörganism an optimum, minimum, and maximum growth temperature, as well as a thermal death point. The optimum temperature is the one best suited to the growth of the microörganism. This varies widely with different species. Northern soils and cold waters contain numerous bacteria, the optimum temperature of which is 15° C. or even lower. Forester, in 1887, obtained from phosphorescent fish light emitting bacteria. These microörganisms not only lived at 0° C. but actually reproduced at this temperature. Since then numerous organisms have been isolated from soil and salt waters which multiply rapidly at 0° C. or slightly lower. To them has been given the name of *Psychrophilic* or cold-loving bacteria. They are of considerable importance in soils of the northern climes, in the deep waters of oceans and lakes, and probably in the changes going on in meats and vegetables in cold storage.

In contrast to the psychrophilic bacteria are certain so-called thermophilic or heat-loving bacteria. These organisms have been repeatedly obtained from the waters of hot springs, the interior of fermenting manure and ensilage, the soil, and the intestinal contents of man and animals. Some of them multiply at a temperature from 70° to 80° C. Setchell found some bacteria living in the water of springs the temperature of which was 89° C. What interesting specks of living protoplasm these must be to live and function at such temperatures. They must be composed of materials far different from those that make up the protoplasm of the ordinary plants and animals; for 89° is near the boiling point, in fact so high is this that it quickly coagulates egg albumen, produces painful burns on the skin, and kills most microbes.

Between the psychrophilic and thermophilic varieties is the group containing the overwhelming majority of bacteria—the mesophilic bacteria. These may be divided into two great classes: (1) Those found in the body of man and animals during health and disease. They have an optimum temperature of about blood-heat (37.5° C.), (2) The saprophitic organisms many of which are essential soil organisms, and have optimum temperatures between 20° and 35° C.

The minimum temperature is the lowest at which bacterial growth will occur. With many of the pathogens this is only two or three degrees below the temperature of the body of the animal which these specific bacteria attack. Some thermophilic bacteria have a minimum temperature as high as 40° C., and some psychrophilic as low as 0° C. or possibly even lower. The maximum temperature is the highest at which growth and multiplication can take place. This in the case of the pathogens is only a few degrees above the optimum, but in the case of the saprophytes it may be many degrees above. For the thermophilic bacteria it may be as high as 89° C., whereas for the disease producers it lies between 40° and 50° C. Certain disease-producing organisms when grown for some time at a high temperature lose their disease-producing power but not their power of calling out anti-bodies; hence, this means is occasionally used in the preparation of vaccines. Most of the psychrophilic bacteria will not grow at a temperature above 30° C.

The temperature relations for the three classes of organisms are: (1) psychrophilic microörganisms—minimum at 0° C., optimum at 15° to 20° C., maximum at about 30° C. To this class belong many of the water microörganisms, especially the phosphorescent bacteria in sea water, and many molds and yeasts. (2) Mesophilic microörganisms reach their minimum temperature at 5° to 25° C., optimum about 37° C., and maximum at about 43° C. To this class belong all pathogenic bacteria, most parasitic, and many saprophytic microörganisms. (3) Thermophilic microörganisms have a minimum temperature ranging from 25° to 45° C., optimum from 50° to 55° C., and a maximum of 60° to 70° C. Some few are able to develop at a temperature of 80° C.

By carefully elevating or reducing the temperature, the limits within which certain bacteria will grow have been altered. The anthrax bacillus grows normally at 37° C. but under certain conditions it has been possible to gradually accustom it to a temperature of 43° C. Pigeons are naturally immune to anthrax, but if given strains accustomed to this high temperature they become infected. Likewise, frogs are immune to normal anthrax, but if given bacteria grown at 12° C., the frogs are killed.

The growth-temperature range for an organism is the number of degrees between the minimum and maximum. This is narrow for pathogens and wide for saprophytes.

Low Temperature.—Although bacteria cannot function below 0° C., they are still very resistant to low temperatures. The

common soil and water organisms as well as the diphtheria and typhoid bacilli have been exposed for days to the temperature of liquid air (about −190° C.) without destroying them. Others have been exposed to the temperature of liquid hydrogen (about −250° C.) with the same results. At this temperature mercury becomes as brittle as cast iron. It is interesting to speculate on what would happen if these bacteria had been cooled down to −273° instead of −250° C. It does not seem probable that this relatively small difference would prevent them from recovering. At −273° (absolute zero) all chemical processes would come to a standstill; hence, if the cell would recover after exposure to this temperature we would have to conclude that cell life may be revived after all chemical activity has ceased within it.

Freezing causes a much greater decrease in the number of bacteria in water than it does in milk. Then too, alternate freezing and thawing is much more injurious than continuous freezing, as may be seen from the following results obtained by Hilliard and his co-workers:

CONTINUOUS FREEZING COMPARED WITH ALTERNATE FREEZING AND THAWING OF BACT. TYPHOSUM

Frozen solid		Alternate freezing	
Before freezing	40896	Before freezing	40896
Frozen 24 hours	29780	Frozen 3 times	90
Frozen 3 days	1800	Frozen 5 times	0
Frozen 4 days	950	Frozen 6 times	0

Crystallization, probably resulting in the mechanical crushing of the bacteria, plays a prominent part in the death of bacteria due to freezing. Moreover, prolonged freezing causes a drying-out of the cell, and we have seen that bacteria cannot continue to live in the absence of moisture. A very interesting condition is often found in natural ice filled with air bubbles. On examining the ice surrounding the air bubble it will be found to contain many more bacteria than does the rest of the block. Curious enough, these organisms are the oxygen-loving ones which have been attracted to this region by the air and then caught on the congealing of the water.

Thermal Death Point.—The bacteriologist recognizes a so-called thermal death point. This is the temperature which under given conditions will kill an organism in a given period of time. It is perfectly evident that a living organism may with-

stand a comparatively high temperature for a short time, whereas a much lower temperature requires a considerably longer amount of time in order to kill. The time, therefore, in determining the thermal death point is usually taken as 10 minutes. One can place his hand in an oven that has a temperature of 130° C. for a short time, whereas if placed for this same time in steam (the temperature of which is 100° C.) a painful burn may be the result. The more effective action of moist heat is due to a number of factors: (1) Moist heat is more penetrating than dry heat; (2) the steam on changing to water liberates large quantities of heat; and (3) death is probably due to the coagulation of the proteins which compose the bacterial body. The temperature at which albumen coagulates varies with the quantity of water present, as may be seen from the following:

Egg albumen + 50 per cent of water coagulates at.....	56° C.
Egg albumen + 25 per cent of water coagulates at.....	74– 80° C.
Egg albumen + 18 per cent of water coagulates at.....	80– 90° C.
Egg albumen + 6 per cent of water coagulates at.....	145° C.
Egg albumen + 0 per cent of water decomposes at.....	160–170° C.

Dry egg albumen may be heated to the point at which it decomposes without coagulation, consequently the thermal death point of microörganisms in water would be much lower than for the same microörganisms in dry air.

The housewife cans strawberries, raspberries, plums, and other acid fruits with considerable ease, but corn and peas often cause trouble. One is acid, the other neutral, and microbes are destroyed more easily in an acid or alkaline solution than in a neutral one. Hence, in determining the thermal death point of bacteria, a neutral salt-solution is used.

Potatoes having on their surface resistant spore-bearing bacteria if dropped into boiling water and left for one hour may still be infected with bacteria. We find, therefore, that the temperature necessary to kill microörganisms also varies with the species and the form in which it is when heated.

Bacteria which do not form spores and the vegetative form of the spore-bearers, which are usually sensitive to heat, are as a rule killed by moist heat at temperatures of 65° to 70° C. The thermal death point for the cholera spirillum is 58° to 60° C.; for the typhoid bacillus 58° to 60° C.; for the vegetative form of the anthrax bacillus 60° C. and for the tubercle bacillus 60° C. If any of the conditions are varied the thermal death point changes.

For instance in covered heated milk, the tubercle bacilli are destroyed in 15 to 20 minutes at a temperature of 60° C. When not covered, a film forms on the surface. Such milk even after being heated for 60 minutes may have living tubercle bacilli in it. The spores of molds can withstand 30 minutes of dry heat at 100° C. but most of them when moist die below 100° C. Bacterial spores can even resist dry heat as great as 140° C., for a considerable length of time although moist heat of about 120° C. for 20 or 30 minutes is enough to kill any bacterial spore.

Light.—Light is essential to the life of higher plants and animals. In its presence plants manufacture their own food from carbon dioxide and water. In the presence of sunlight animals can utilize, if not actually manufacture, vitamin D more effectively than they can in its absence. However, the direct rays of sunlight may be injurious to living tissue. This fact is well known by all who have ever gone boating or fishing after long confinement indoors. Bacteria are even more sensitive to light than are the cells of the human body; for diffused sunlight hinders their growth, and the direct rays of the sun are highly injurious. Pathogens are more sensitive than saprophytes. Some of the disease producers are killed almost instantly when exposed to the sun's direct rays. On this rests the foundation of the oft-quoted proverb: "Where the sun does not enter, the doctor does." The sunlight's effectiveness varies not only with the organism but with the specific light rays and the medium in which the microörganisms are suspended. Only the ultra-violet, violet, and blue rays of the spectrum possess bactericidal action; green light has very much less power; red and yellow none at all. The germicidal power is greatest in (and some workers actually believe confined to) the ultra-violet regions of the spectrum. These parts of the spectrum are more active against organisms in boullion than in milk, and in a medium like soil the influence of the light is confined to the very surface. The action of light is probably due to chemical changes which are produced in the bacterial protoplasm or the medium in which the organism is suspended, as sunlight is without effect upon bacteria in an atmosphere of carbon dioxide. Bacteria grow poorly on a medium which has long been exposed to the action of light before seeding. This indicates that sunlight may produce toxic substances in and from the media.

The movement of some bacteria may be influenced by light. Engelmann found that if a microscopic preparation of *Rhodo-*

spirillum photometricum be illuminated in such a manner that the light rays can fall only on one sharp definite portion, then, all of the roving bacteria collect within this space and move about briskly. If one of them in its onward career passes beyond the circle of illumination into the dark portion, it stops instantly and returns to the illuminated field. This Engelmann called the movement of alarm. Consequently, each sharply defined

FIG. 40.—Thickly sown plate culture of typhus bacilli on agar-agar. Covered with paper letters and exposed to the sun's rays for one and a half hours, then kept twenty-four hours in the dark, whereupon development of thickly congregated whitish colonies was found only at the parts covered by letters. (*After H. Büchner.*)

illuminated portion of the field acts as a trap for the bacteria, from which they cannot escape until the illumination has been altered.

Sunlight probably plays a major rôle in the sterilization of water and the destroying of pathogens which find their way into the air during coughing, speaking, and sneezing. Büchner, who was the first to appreciate the value of light in this respect, forcefully illustrated it by pouring bouillon agar inoculated with

a copious supply of typhoid bacilli, into Petri dishes on the underside of which were affixed the letters *TYPHUS*, cut out of black paper. The dishes were exposed to the sun's rays for a period lasting from one to one and a half hours (or to diffused sunlight for two hours), and afterward incubated in a dark room for twenty-four hours. On removing the paper letters, a growth of the bacteria which had been protected by the paper from the sunlight stood out boldly.

Other Rays.—X-rays and radium emanations have little effect when allowed to play directly upon bacteria. However, when the bacteria are in living tissue they are often destroyed. This is probably due to a reaction of the stimulated tissue and not to the direct action of the various rays.

Electricity.—Can the little microbe like man be shocked to death by the electric current? It would seem, from the work which has been done, that this is not possible. The passing of the electric current through a medium in which bacteria are growing destroys many of them, but this is not due to the direct action of the electricity, but rather to the heat generated and the chemicals produced in the medium as the current passes through it. The electric light kills bacteria if it is permitted to act on them long enough, but they are much more resistant to this than they are to sunlight.

Electricity is used quite extensively tody in the sterilization of water and sewage, and some have suggested its use in sterilization of milk and other food products. However, the expense involved precludes its extensive use at the present time.

Shaking.—When subjected to prolonged shaking bacteria are first prevented from multiplication and later killed. This may be due to the actual breaking to pieces of the cell by the violent agitation. However, it is well-known that continuous shaking of some proteins will cause them to coagulate.

Osmotic Pressure.—If a perfume is liberated in one part of a room its odor can soon be detected in any part. We, thus, find that gases tend to diffuse from points of high to points of low concentration. Similarly, if we dissolve copper sulfate in water, place it in the bottom of a tall cylinder, and cover this solution with pure water, we shall find, as time goes by, that the blue zone of copper sulfate creeps up into the clear solution and that eventually the two solutions will be of uniform concentration. The force which drives the copper sulfate from the point of high concentration to that of low is known as diffusion pressure, and

is found to be dependent upon the number of particles of the substance in solution. By appropriate means this pressure can be measured and is the osmotic pressure. We have learned that a cell is covered by a membrane which readily allows water to permeate it but salts can only do so slowly. Furthermore, we have learned that the concentration of protoplasm in the cell is about the same as a 0.9 per cent solution of common salt. A cell dropped into such a solution neither loses nor gains in weight, but if it is dropped into a more concentrated salt solution it shrinks. A careful microscopic examination of such a cell shows that the inner ectoplasm has been torn from the cell wall. This is called plasymolysis. The salt solution bathing the cell is more concentrated than the solution found on the inside. The salt molecules cannot get in to equalize the pressure; hence, the water passes out through the cell membrane to dilute the solution. The plasmolysis of a cell may cause its death. It is because of these peculiarities that the placing of bacteria into strong salt or sugar solutions usually causes their death. Advantage is taken of this in the making of jellies, syrups, and preserves. Moreover, it is one of the factors which render alkali soil barren. We find that when the osmotic pressure of the soil reaches about 8 atmospheres the activities of the nitrifiers are decreased, and are entirely stopped when the pressure has reached from 15 to 20 atmospheres. The ammonifiers are interfered with when the osmotic pressure reaches 9 atmospheres, but they are not entirely stopped in their actions until a pressure from 25 to 30 atmospheres has been attained. The nitrogen-fixing organisms are even more resistant to osmotic changes than are the ammonifiers.

Pressure.—Bacteria are extremely resistant to pressure, for they are found at depths in the ocean where the pressure is tremendous. However, successful attempts have been made to preserve fruit and vegetables by exposing them to high pressure. Apple juice subjected to a pressure of 4,000 to 8,000 atmospheres for thirty minutes did not develop gas later. Peaches and pears exposed to this pressure did not spoil for five years. However, those vegetables on which resistant spores occurred could not be preserved by pressure.

Poisons.—The action of poisons upon bacteria varies with the microbe, the kind of poison, and the conditions under which the poison is used. Extremely weak solutions of most poisons stimulate bacteria, a stronger concentration prevents growth and

multiplication, whereas a still more concentrated solution may kill them.

A substance which kills microörganisms is called a germicide; a disinfectant is a substance used to destroy pathogens. However, if it only prevents growth and activity it is known as an antiseptic. The application of a disinfectant as a gas is termed fumigation. When a chemical is added to a food to prevent decay it is called a preservative. In sanitation disinfectants and antiseptics are used extensively in the prevention of disease. In agriculture they are often used on plants, or in the soil, to destroy injurious microbes. The principles involved in both cases are similar and are subject to certain general laws.

(1) The efficiency of a disinfectant varies with the amount of moisture present. A dry poison has but slight action upon bacteria. Dry formaldehyde, or sulfur dioxide, is practically without effect. In a similar manner absolute alcohol has not nearly the germicidal power of 60 to 70 per cent alcohol. This is probably due to the coagulation of the outer membrane of the organism by the absolute alcohol thereby preventing the poison from diffusing into the vital parts.

(2) The temperature of the medium in which the organisms are present materially influences the action of a disinfectant. A 5 per cent solution of common soda at normal temperature may have but little germicidal influence, but if the temperature of the medium in which it is placed is raised to 40° C. then it becomes quite effective.

(3) Bacteria are more easily killed by a disinfectant in pure water than in saliva, blood, or other body fluids.

(4) Emulsions usually have greater germicidal effects than true solutions. This is due to the fact that the emulsion has little globules of the concentrated poison distributed throughout the solution, and if examined under the microscope these, together with the suspended bacteria, will be seen moving about in the solution. When the bacteria get into one of these highly concentrated drops of poison they are quickly killed.

(5) Disinfectants of the heavy metal group in order to be effective must be in solution so as to permit the formation of the ions which combine with the protein of the bacterial cell and thus cause death.

(6) The action of a disinfectant varies with the time. Even a fairly weak poison acting for a long time will cause death.

Some of the more extensively used disinfectants are quicklime, bleaching powder, sulfur dioxide, formaldehyde, and mer-

curic chloride. That extremely violent poison, hydrocyanic acid, of which a Freshman once said, "It is so poisonous that a drop placed upon the tongue of a dog will kill a man," is a very poor germicide, but it is an excellent insecticide.

Chemotaxis.—It has been repeatedly shown that bacteria, like other free moving organisms, are attracted by certain chemical substances in solution and repelled by others. The attraction of a motile organism by a chemical is *positive chemotaxis*, the repulsion *negative chemotaxis*.

A very simple and efficient method of studying this characteristic of bacteria is as follows: A capillary tube from 5 to 10 mm. in length and sealed at one end is filled with a 5 per cent slightly alkaline solution of beef tea, or peptone. The outer surface of the glass is carefully cleaned from any trace of the beef tea and the tube is then placed in a drop of water containing bacteria. In a few seconds the bacteria are found to thickly congregate around the open end of the tube. This is not due to any volition on the part of the bacteria, nor is it due to their congregating in places most favorable for life; for had the capillary tube been filled with sugar or glycerine, which are excellent food-stuffs, there would have been no such gathering of the bacteria at the end of the tube. Moreover, a dilute solution of potassium chloride and mercuric chloride attracts bacteria, but they rush into the tube only to meet their death. Some say that bacteria are attracted to the roots of the alfalfa in the soil by some such means, and it is quite certain that the white corpuscles are attracted to an infected wound by this means.

Reaction.—All living cells are influenced by the reactions of the medium in which they are living. Most bacteria require a neutral or slightly alkaline reaction for optimum growth, whereas yeast and molds will tolerate and, in some cases, are actually favored by acidity. Hence, the microflora growing in any natural medium is governed by the reaction of that medium. Bacteria predominate in normal soils, whereas in acid or alkali soils there are a great number of molds. As lime or limestone is added to an acid soil and as an alkali soil is leached, the molds become fewer and the bacteria more numerous. Bacteria rapidly multiply in fresh milk until the acidity reaches a certain limit, after which they are replaced by molds.

Reaction, in any medium which is the relative acidity, and alkalinity, may be expressed in two ways: (1) By the number of cubic centimeters of normal acid or alkali required to bring the

material to the neutral point of some indicator. This merely indicates the titratable acid or alkali and may or may not represent the neutral point, depending upon the specific indicator used and the medium being titrated. Proteins, for instance, may give a high titrative number and have a low acidity or alkalinity. In the past this has been the principal method used to determine acidity or alkalinity by the bacteriologist. (2) By the true hydrogen-ion concentration, or conversely the true hydroxyl-ion concentration of the medium. This may be determined by either conductivity methods or by the use of specific standardized indicators.

Acidity is determined by the presence of free hydrogen-ions, and alkalinity by the presence of free hydroxyl-ions in a given volume. A solution is truly neutral when it contains an equal number of hydrogen and hydroxyl-ions. Pure distilled water is neutral, and analyses have shown that it contains approximately 0.0000001 gram of hydrogen-ions in a liter. This may be written: 10^{-7} grams hydrogen-ions per liter. A normal solution of hydrogen is one which contains one gram of hydrogen-ions in one liter. Hence, a neutral solution is one which is 10^{-7} normal. Inasmuch as a neutral solution contains equal numbers of hydrogen and hydroxyl-ions, it must also contain 10^{-7} grams of hydroxyl-ions. The chemist has discovered that the normality of the hydrogen-ion concentration times the normality of the hydroxyl-ion of any solution is equal to a constant-approximately 10^{-14}. Therefore, if the hydrogen or hydroxyl-ion concentration of any substance be known, the unknown can be readily calculated. If the hydrogen-ion concentration of a solution be 10^{-4}, the hydroxyl concentration would be 10^{-10}. Using this information we may make the following scale to indicate the acidity of solutions:

$$10^{0},\ 10^{-1},\ 10^{-2},\ 10^{-3},\ 10^{-4},\ 10^{-5},\ 10^{-6},\ 10^{-7},\ 10^{-8},$$
$$10^{-9},\ 10^{-10},\ 10^{-11},\ 10^{-12},\ 10^{-13},\ 10^{-14}.$$

That is, the larger the numerical value of the exponent the smaller the hydrogen-ion concentration and the greater the hydroxyl concentration. Such numbers are somewhat cumbersome; hence, Sorensen suggested that only the exponent be used, and this be taken as a positive number. This he designated the Ph, hydrogen potential, of the solution. Written in terms of the Ph values, our scale becomes:

0		8
1		9
2		10
3		11
4		12
5		13
6		14
	7	

All values to the left of the line indicate an acid solution, and the smaller the number the greater the acidity. All numbers to the right of the line indicate an alkaline solution and the larger the number the greater the alkalinity.

CHAPTER XIII

THE CARBON CYCLE

Matter is indestructible; it can be neither generated nor destroyed by any means known to man. He can change it from one compound into another, and varied are the forms in which he can make it appear. It has existed since the birth of the world and the rôles which it has played have been numerous indeed. The same bit of phosphorus, carbon, nitrogen, or other chemical element has appeared at one time in the apparently indestructible rocks of the everlasting hills, a little later in the body of a plant, and then in the body of a lower animal. Still later this same bit of matter may have formed a vital part of the body of a man. Plant, animal, or man may have been devoured by bacteria, and this same bit of material was returned to the soil to commence once more its wonderful journey through the bodies of plants and animals. In these transformations from the complex to the simple, from the simple to the complex, from the living to the dead, and from the dead to the living, bacteria play a very prominent rôle.

Carbon.—The element carbon is well represented in hard coal. Soft coal and charcoal are chiefly carbon. The diamond is pure crystallized carbon, and charcoal made from pure sugar is pure uncrystallized carbon. Carbon has certain outstanding properties; it has a valence of four and readily combines with itself and other elements, thus yielding a great variety of interesting and valuable compounds. There are at the present time approximately 250,000 compounds of carbon known, and the possibility of new compounds is limitless. Moreover, "carbon is the element par excellence, whose absence from any chemical substance stamps this forthwith, by common if somewhat arbitrary consent, as inorganic: whose presence affords the soil and season for the growth of what might be termed the tropical jungle in the domain of chemistry. For in the compounds of carbon nature seems to have run riot, in a revel of creative versatility, as if trying to set a record unapproached elsewhere in all the realm of chemistry, for number, variety, and complexity of her children. Other ele-

ments—oxygen, nitrogen, phosphorus, sulfur, iron—indeed play a significant rôle in life processes; but the indispensable bond that ever links all other ingredients in organic unity is carbon. Furthermore, carbon is preëminently the energy carrier, the standard coin of the organic realm, for in it both the first cost of installation, of anabolic tissue building, and also the running cost of operation, of metabolism, is defrayed."

Occurrence of Carbon.—Carbon is widely distributed in nature. It is found in the earth in a pure form as diamonds and graphite. It occurs in the earth as coal to the extent of over five hundred billion tons. Chemically combined, it exists in far larger quantities in limestone, chalk, marble and dolomite. It is estimated that 0.19 per cent of the entire earth is carbon and that sedimentary rock alone contains 30,000 times as much as occurs today in the atmosphere. The atmosphere contains about 3 parts in 10,000, which is equivalent to six hundred billion tons of carbon. There is as much more in living things as there is in the atmosphere, and according to Clark from 18 to 37 times this quantity in the ocean. According to Pettenkofer, a man weighing 154 pounds contains 26.4 pounds of carbon; thus, no less than two hundred and fifty-seven million tons of it is stored up in the bodies of men and women living upon the earth at the present time, in addition to the far greater quantities occurring in the tissues of plants and lower animals.

While the earth was in the molten state carbon occurred in the atmosphere as carbon dioxide. As the earth cooled this was absorbed and when the temperature dropped so that water could exist upon it, water also drank in the carbon dioxide from the atmosphere. The huge primitive plants which covered this earth in early geological ages were potent factors in removing it from the atmosphere, and as the carbon dioxide of the atmosphere decreased climatic changes occurred, until there resulted our present condition.

The Cycle of Carbon.—There are two great factors at work removing carbon from the atmosphere: (1) The decomposition of carbon dioxide by plants with the liberation of oxygen and the production of organic compounds; (2) the consumption of carbon dioxide in the weathering of rocks. No precise valuation can be given to either of these factors, although various attempts have been made to estimate their magnitude. Cook considers that leaf action alone more than compensates for the production of carbon dioxide. Chamberlain estimates that the amount

of carbon dioxide annually withdrawn from the atmosphere is 1,620,000,000 tons, the greater part of which is taken up by the weathering of rocks.

It is being continually added to the atmosphere by several processes: the combustion of fuel, the respiration of animals, and the decay of organic matter. Enormous quantities are being evolved from mineral springs and volcanoes. The magnitude of this factor can be seen in the statement "that if volcanoes became extinct it would not be long before the existence of life would be impossible due to a lack of carbon dioxide."

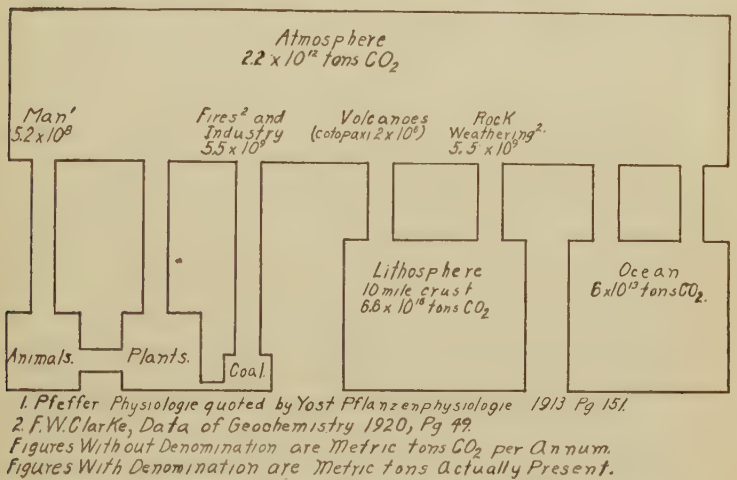


FIG. 41.—Illustrating carbon cycle in nature. (*After Lotka.*)

Krogh estimates that the annual consumption of coal adds yearly to the atmosphere about one-thousandth of its present carbon dioxide content. Hence, there are two compensating sets of factors continually at work in nature: (1) Plant growth and rock weathering which is taking carbon dioxide and building from it more complex compounds. (2) Decay, respiration and combustion which are continually returning it to the atmosphere. These two sets of processes nearly balance each other, thereby completing the carbon cycle and rendering the carbon dioxide content of the atmosphere nearly constant.

Carbonation, which is the union of carbon dioxide with a base, plays an important part in the removal of carbon dioxide from the atmosphere. A familiar example of this is the hardening of

plaster due to the combination of lime with carbon dioxide and the formation of limestone. In nature many minerals take up carbon dioxide. This changes their volume and often their solubility, and as a result the rocks fall to pieces. This process increases the solubility of the potassium and the phosphorus of the soil. Högbom points out the importance of the carbonation process by stating that it nearly balances the regeneration of carbon dioxide resulting from the burning of coal. Its accumulated record is found in the sedimentary rocks which contain approximately 30,000 times as much carbon dioxide as is today found in the atmosphere.

Photosynthesis.—Plants absorb water from the soil by means of their roots. This passes upward through the stem, the petiole, and the veins, and thence enters the main portion of the leaf. Here it comes in contact with carbon dioxide which has been taken in from the air. Although the quantity in the atmosphere is only three parts in ten thousand, yet it is effectively picked out by the green leaves, as a single large tree may expose a leaf surface of more than an acre, and the surface which absorbs carbon dioxide is much greater than this. In the presence of chlorophyll and under the influence of sunlight the water is caused to combine with carbon dioxide. Oxygen is given off. Probably the first product formed is formaldehyde. This is a poison and is not permitted to accumulate. The first product which does accumulate in sufficient quantities to be detected is glucose or grape sugar. This is present in the sap of practically all plants, and ultimately from it are produced all the varied organic compounds of the plant and animal kingdoms. The process of photosynthesis is fundamental in organic nature, for not only is it an essential function of green plants, but it is vital to animals and man; because (with the exception of the small quantity of carbon fixed by a few species of bacteria) it constitutes the source of food for all living organisms.

Attempts have been made to compute the quantity of carbon fixed by plants, but these can only be taken as approximations. One of the earliest attempts was made by Liebig. He considered the annual output of dry organic matter of central Europe to be one ton per acre. If 40 per cent of this were carbon, there would be an annual total production, by plants, of 13,000 million tons of carbon. This is nearly ten times the world's annual coal consumption and about one-fifteenth of the total carbon in the atmosphere. The botanist, Yost, com-

putes that if the entire land area were planted to sunflowers, approximately 6.5×10^{11} tons of carbon dioxide would be annually absorbed; whereas, Arrhenius points out that if all the carbon annually fixed by plants were deposited in peat bogs, the atmosphere would be depleted in half a century. However, this does not occur, as there are many factors liberating carbon dioxide.

Production of Carbon Dioxide.—Probably one of the greatest sources of atmospheric carbon dioxide is from volcanoes and mineral springs. Cotopaxi alone has been credited with an annual discharge of two million tons of the gas.

From every chimney of every factory and home where gas, oil, wood, or coal is burned, carbon dioxide is being returned to the atmosphere. The breath of every living animal is laden with it. In reality, all the coal that is mined, all the trees that are cut for fuel, all the food that is used for energy by man and the lower animals pass into the atmosphere as carbon dioxide. The magnitude of each can be only roughly measured. It is estimated that from coal fires and metallurgical furnaces alone ten times as much carbon dioxide is returned to the atmosphere annually as by the biological process of breathing. Man alone in the course of five hundred years would throw off enough carbon dioxide to double that in the atmosphere were there no compensating factor removing it. The forests are being burned and the deposits of peat and coal evaporated. During one season huge quantities of carbon dioxide are poured into the atmosphere. During another season equally large quantities are removed; yet, the carbon dioxide content of the atmosphere remains nearly constant. This is due to the fact that the ocean acts as a great buffer, at one time giving off, at another time drinking in carbon dioxide. Thus of every 2,000 pounds of carbon dioxide thrown into the air by volcanoes, fires, and animal respiration, the ocean ultimately receives directly or indirectly about 1,900 pounds, leaving only the balance of 100 pounds in the atmosphere.

As outlined above, there are certain leaks in the carbon cycle, and were this the entire process, the carbon in the bodies of plants and animals, which are not consumed by fire, would be permanently locked up. But this is not all. Plants and animals (including man) are permitted to keep their bodies as long as they are useful, but just as soon as life becomes extinct they are dragged off to the bacterial bonfire, so that the elements composing them can serve other plants and animals.

Decay.—During the process of decay, carbon and hydrogen are liberated as water, carbon dioxide, methane, and other volatile products, with the result that the carbon in the medium tends to decrease relatively to the nitrogen; hence, the carbon-nitrogen ratio becomes narrow as compared with the fresh material. In soils the ratio of carbon to nitrogen indicates the nature of the organic matter and the extent to which it has decomposed. The ratio of carbon to nitrogen in green plants is 25–40:1, whereas, in field humus it is 10–15:1. Legumes and other materials rich in nitrogen yield a residue in the soil with a narrow carbon-nitrogen ratio. Straw, leaves, and similar substances give a wide ratio. The further the decay has progressed in a soil, the narrower the carbon-nitrogen ratio. Other things being equal, a wide carbon-nitrogen ratio indicates a more fertile soil than one containing a narrow ratio.

Humus.—The plant residues which find their way into soil are rapidly disintegrated by bacteria, and there results a light mass of partially decayed black organic matter—the so-called humus. This is the storehouse of the soil nitrogen, and as it is decomposed by bacteria the nitrogen is liberated and transformed into nitrates. During the transformation carbon is liberated in relatively greater quantities than is nitrogen, with the result that the carbon-nitrogen ratio becomes narrow and the resulting mass more resistant to bacterial activities. Its composition and value depend upon the material from which it was produced. Proteinaceous materials yield a substance rich in nitrogen. The rapidity with which it is produced also varies with the original material. All of the simple carbohydrates and many proteins are quickly attacked by numerous bacteria, whereas nucleoproteins and cellulose are more resistant and are decomposed by fewer species of microörganisms. Humus renders the soil light and porous, thus increasing its water-holding capacity and rendering it more easily tilled. However, the greatest effect upon the chemical and biological transformations of the soil has been exerted before the carbonaceous material reaches the stage of humus.

Part of the humus is worked over by soil fungi and incorporated into their bodies, but the greater part of it is slowly oxidized with the formation of a coal-like residue which remains as an inert mass in the soil.

Cellulose.—The woody fiber which constitutes the cell wall of plants is a very resistant carbohydrate, cellulose. Next to

water it is the most abundant substance in the vegetable world. It is to the plant what bone is to the animal. It is especially abundant in the roots, stems, and leaves of mature plants. It constitutes from 30 to 50 per cent of hay and coarse fodder. Straw, husks, and old mature plants contain much larger quantities. Cotton, linen, and paper are examples of nearly pure cellulose. It is insoluble in all the common solvents and is resistant to most chemicals. Cellulose dissolves in ferric chloride, sodium hydroxide, and carbon bisulfide and is broken down by various acids into simpler soluble substances.

Early Observations on Cellulose Fermentation.—Cellulose finds its way into the soil in large quantities in the residues of plants, and early in the history of the race man must have observed that it slowly disappeared. But it was not until the time of Pasteur that microörganisms were considered as being the cause. He considered that cellulose was decomposed by molds. Later Mitscherlich (1850) observed that as moist potatoes decay the cell wall is dissolved and the starch gradually passes out. He believed this due to the action of microörganisms, but nothing was done to show that it was the work of any species until about fifteen years later when Trécul isolated an organism which had the power of decomposing young plant tissues and which was stained blue by iodine. He gave to this organism the name amylobacter. He claimed the organism had the power of decomposing cellulose with the formation of butyric acid, carbon dioxide, and hydrogen. All of his work was carried on with plant tissues; hence, it left a question as to whether the amylobacter had actually decomposed cellulose or only some of the nearly related compounds. Hoppe-Seyler was one of the first to work on the change through which cellulose passes in soil. He commenced his experiments by collecting and analyzing the gases given off from soils and swamps. These were found to consist mainly of carbon dioxide and methane. Later he studied in his laboratory cellulose decomposition by placing 25.773 gm. of filter paper in 1,000 cc. of water inoculated with mud. He kept the fermenting mass at room temperature for four years and collected the resulting gaseous products over mercury. The first year there was considerable gas evolved, but the evolution gradually became slower, until at the end of four years it had practically ceased. An analysis showed that 15 gm. of the cellulose had been decomposed with the formation mainly of carbon dioxide and methane. He was unable to find any of the

true sugars, although he thought it possible that there was some of the dextrin compounds in the solution. When air was excluded there was a greater production of methane and a smaller production of carbon dioxide. From his work he concluded that the reaction proceeded in two stages: (1) The cellulose is caused to take up water and form a six-carbon sugar. (2) This sugar is broken into carbon dioxide and methane, or perhaps acetic acid is first produced with carbon dioxide and methane resulting from the splitting of this.

Later workers studied the decomposition of cotton, manure, straw, and filter paper, and always found the same results—that there were produced considerable quantities of carbon dioxide,

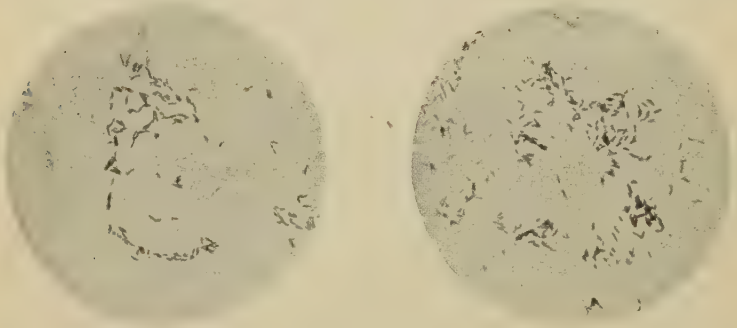


FIG. 42.—Cellulose ferments. (*After McBeth.*)

other gases, and various acids. The quantity and quality of each varied with the fermenting substance and the material which was added to it.

The evidence was strong that cellulose decomposes under anaërobic conditions with the production of gases and acids, but nothing definite was known concerning the causative organism until toward the close the of nineteenth century, when a Russian investigator, Omelianski, described two spore-bearing anaërobic organisms which slowly decomposed cellulose. One of them attacked it with the production of hydrogen, the other with the production of methane. Both produced fatty acids and carbon dioxide. It is probable that these organisms operate only in soils under conditions of inadequate aëration, for it has been shown that these gases are evolved only from swamps and rice soils, and that the methane and hydrogen are oxidized by other microörganisms.

In normal soils the decomposition takes place in the presence of oxygen and follows a far different course, as shown by the discoveries made somewhat later by Kellerman and McBeth, who isolated the true cellulose ferments from the soil. These ferments were aërobic and much more active than those studied by earlier workers. All were rods varying in length from 0.8 to 3.5μ. Involution forms were observed for only three species. Five species were found to produce spores; twenty-seven were motile. Of these, seven were pseudomonas and twenty-three bacilli. It was shown that a few were facultative anaërobes, but most of them true aërobes. The optimum temperature lies between 26° and 33° C. They grow rapidly on solid media such as beef agar, gelatin, starch, and potato. Nineteen species liquefy gelatin. They all decompose cellulose and other carbohydrates with the production of acids. Not one produces a gas.

In 1919 Hutchinson and Clayton described a remarkable organism which they isolated from Rothamsted soil and which they named *Spirochaeta cytophaga*. It is a true aërobe. In the active stage it exists for the most part as a thin flexible rod tapering at the extremities. This form passes into a spherical cyst-like stage. It appears to be unable to derive energy from any carbohydrate other than cellulose, and curious enough in pure cultures all the simple carbohydrates appear to be toxic to the organism.

The action of bacteria upon proteins yields amino-acids. These upon further decomposition yield ammonia and nitrogen-free compounds. Among these nitrogen-free products are carbolic acid and related compounds. These substances in time, if permitted to accumulate in the soil, would become injurious to plants including the microflora of the soil. Recent work at Rothamsted has shown that numerous soil microörganisms possess the remarkable power of utilizing these compounds as their sole source of energy.

Function of the Cellulose Ferments.—The cellulose ferments are especially valuable in liberating the carbon which finds its way into sewage in the form of cellulose. In the septic tanks there are millions of these microörganisms, busily changing this resistant organic material into gaseous products, and today many large cities depend upon them for the disposal of their waste. They also play a great part in the purification of a city's water supply. Omelianski considers them of prime importance in the formation of soil humus, for he writes, "It is possible that a general reaction of this sort forms the basis of the

universal processes of humification, that is, the gradual transformation of organic substances into mixtures of brown and black substances with a high content of carbon, such as is characteristic of fossil coals. But whatever the mechanism of these transformations, the active participation of microörganisms in the latter cannot be denied."

The cellulose ferments break the plant residues into less complex organic compounds which are decomposed by other microörganisms with the generation of large quantities of organic acids. These acids react with the minerals of the soil and render them available to higher plants. This very likely explains the beneficial results obtained when raw rock phosphate and stable manure are used on phosphorus-poor soil. The fermentation of the cellulose yields acids which render soluble the phosphorus. However, the production of acids may at times become excessive, thus giving rise to the sour humus of moors and heaths.

It is well-known that the fermentation processes in the soil resulting in the decomposition of organic matter may give rise to large quantities of carbon dioxide, methane, and hydrogen. The hydrogen and methane do not all pass into the atmosphere, but, according to the researches of recent investigators, furnish energy to numerous other soil microörganisms. The importance of this however, still remains almost wholly for future workers to develop. The first work on this subject was done by Immendorff who, in 1892, found that hydrogen and oxygen can be united under the influence of soil. He found that the oxidation of hydrogen was brought about only by normal soil and not by soil previously treated with chloroform vapor. This observation remained unnoticed until two papers appeared—one by Kaserer and the other by Solmgen—which threw considerable light on this phase of carbon and hydrogen transformation. These men used an inorganic solution containing dipotassium phosphate, ammonium chloride, magnesium sulfate, sodium bicarbonate, and a trace of ferric chloride. This they inoculated with a small quantity of soil and confined it in an atmosphere consisting of a mixture of hydrogen, oxygen, and carbon dioxide. Growth took place, and the hydrogen disappeared. The presence of a small quantity of carbon dioxide seemed to be necessary for the development of the microörganisms. It also appeared that like the nitrifying bacteria the hydrogen ferments can produce bacterial proteins in inorganic solutions, deriving their carbon from carbon dioxide. This reaction, according to Lipman, is of

great significance in agriculture, for a great quantity of energy is conserved through the bacterial oxidation of hydrogen which has been produced in the deeper layers of the soil by anaërobic ferments. It also partly counteracts the rapid mineralization of organic materials in that it leads to the formation of complex compounds from carbon dioxide, hydrogen, and oxygen.

The cellulose ferments also perform direct beneficial functions in the soil, for instance in the liberating of plant-food which is bound up in the residues of plants. Heinze has attributed to bacterial activities much of the benefits resulting from summer-fallowing. He found in quantitative studies that bacteria were more numerous in fallow soil than in cropped soil. He thinks the benefit comes from their activities in rendering the cellulose more suitable as a carbon supply for the Azotobacter, thus causing the increase of soil nitrogen in fallow land noted by a number of recent workers. Hence, they are the organisms which decompose the cellulose of straw so it can be utilized by the Azotobacter in the fixation of atmospheric nitrogen. More recent investigators have shown that under suitable conditions the Azotobacter may fix 325 mgm. of nitrogen for every 100 gm. of straw decomposed. At this rate there would be a gain of seven pounds of nitrogen for every ton of straw utilized. This, in addition to other beneficial effects, the farmer loses when he burns his straw. The cellulose ferments are being used in the synthetic production of manure. Investigators at Rothamsted have shown that if a straw heap is treated with the correct proportion of ammonia and inoculated with cultures of *Spirochaeta cytophaga* it changes into a substance having the appearance of a well-rotted manure. Field tests of the product shows that it produces an effect very similar to that of natural barnyard manure. Recently an attempt has been made to use cellulose ferments in the production of acetic acid. A mash of cellulose material (such as sulfide pulp or straw) is inoculated with fermenting vegetable material such as stable manure and maintained under aërobic conditions at a temperature between 25° and 60° C. The cellulose undergoes acetic fermentation, and calcium carbonate, or some other suitable substances are added to neutralize the acid as it is produced. Later the acid is separated from the fermenting mass and purified. Some day probably this process will be improved upon and very active pure cultures used.

CHAPTER XIV

THE NITROGEN CYCLE

It is carbon that stamps a compound as organic, but it is nitrogen that determines the extent of life on this planet. The whole atmospheric ocean of carbon dioxide is available to growing plants, but only the small quantity of nitrogenous compounds within feeding area of their roots is available. Carbon dioxide is utilized by all green plants, free nitrogen by a few and then only in the presence of microörganisms.[1] It is thus seen that bacteria play a greater part in the nitrogen cycle. This cycle has been given much more consideration by investigators than the cycle of the other elements.

Occurrence of Nitrogen.—Elementary nitrogen constitutes seventy-nine per cent of the atmosphere. It is contained in the mineral matter of the earth only in minute quantities, but enters into the structural material of all plants and animals. Consequently, it reaches the soil as a part of their bodies, and becomes incorporated into its surface. This is the source of nitrogen for growing plants.

"The proportion of nitrogen to carbon in the human body is 1:2, in the atmosphere it is 5,500:1. A human adult contains in his body about twelve pounds of nitrogen and over twenty-five pounds of carbon. Over every square foot of the earth's surface rises a column containing some 1,500 pounds of nitrogen, and only one-fourth pound of carbon. The demand and supply of these two elements appear therefore at first sight, to be altogether out of all proportion favorable to nitrogen. Yet, in point of fact, the practical problem of securing an adequate supply for the sustenance and expansion of life is incomparably more complex in the case of nitrogen than in the case of carbon. The reason for this somewhat remarkable inversion is to be seen in the fact that nitrogen is readily accessible as food for living

[1] Recently C. B. Lipman and J. K. Taylor have shown that some plants can under appropriate conditions assimilate small quantities of nitrogen in the absence of bacteria, but this does not modify the economic importance of this statement.

organisms only when it occurs in certain chemical combinations, and nitrogen thus combined is far from plentiful."

Properties of Nitrogen.—Pure elementary nitrogen is a colorless, tasteless, odorless gas. It is distinguished primarily by negative properties. It will not support combustion, nor will it support animal life. It is not poisonous, just inactive. It combines directly with only a few of the other elements, but can be indirectly made to combine with many and in this way yield numerous interesting compounds possessed of remarkable characteristic properties. It is incombustible under ordinary conditions, but under the influence of a powerful electric current it can be made to combine with oxygen. The combustion, even under this condition, does not extend to the surrounding air, for heat is absorbed and must be supplied from some external source.

A world whose atmosphere consisted solely of nitrogen could not support life even for a few minutes. "The dead listless air would choke out their lives almost immediately not because nitrogen is in any way poisonous in the sense that coal gas is, but merely because it is so chemically indifferent that it cannot support the combustion required for our lives. Men and animals require oxygen and are indifferent to nitrogen.

"Perhaps, however, the most extraordinary thing of all on such a planet would be the incombustibility of everything which burns freely in the atmosphere of our earth. No matter how hard we tried, no candle nor oil lamp could be lighted, and paraffin oil could be poured upon white hot coal without catching fire! Indeed, the oil would only serve to quench the heat of the coal just as water would! Our cheerful coal fires would be an impossibility on such a planet, for coal would be as incombustible as gold or stone. In fact, it would be a perfectly useless mineral, and not, as on our earth, the source of untold wealth and power. It is true that by its destructive distillation gas could be obtained, but as this would be as incombustible as nitrogen itself, it too would be quite useless."

Although in the free form nitrogen is inert, in the combined form it is very active. The compounds of nitrogen are extraordinary. The most beautiful dyes contain it. It is tucked away in our most powerful explosives. It enters into the composition of our most delicate perfumes, and it is found in the vilest smelling compounds. It plays its part in our most useful drugs, it is contained in the most powerful poisons; and, most wonderful of all, it is the basis of living protoplasm.

Proteins.—Most of the nitrogen-carrying compounds composing the bodies of animals and plants, including bacteria, are proteins. They are also the principal nitrogenous compounds decomposed by microörganisms. For these reasons it is requisite that the student have at least a nodding acquaintance with a few of the fundamentals of protein chemistry.

Proteins are complex organic compounds composed of the elements carbon, hydrogen, oxygen, nitrogen, and usually sulfur. In addition to these some proteins contain phosphorus, iron, copper, iodine, and even other elements. The quantity of the various elements in the different proteins varies between comparatively narrow limits, as may be seen from the following:

Elements	Per cent
C	50.6 to 54.5
H	6.5 to 7.3
N	15.0 to 17.6
O	21.5 to 23.5
S	0.3 to 2.2
P (when present)	0.4 to 0.9

Viewed from the chemical standpoint a protein has a huge molecule, complex in structure, unstable in character, and hence prone to chemical change. So large and intricate is the protein molecule that chemists for generations have been baffled in their attempts to gain an adequate conception of its nature. Some conception may be gained of the size of the molecule from its calculated molecular weight of approximately 16,000 and its complexity from the formula $C_{239}H_{389}O_{78}N_{58}S_2$, which has been suggested for one of the proteins in white of egg. Many of the proteins are soluble in water, salt solutions, or alcohol in which they occur as colloids, in which condition they do not pass through parchment paper.

Numerous natural proteins occur in both plants and animals. These are divided into groups, depending upon the complexity of their structure and their solubility in various solvents—pure water, saline solutions, alcohol, and their like. They are decomposed by means of acids, alkalies, superheated steam, and the enzymes secreted by plants and animals, and yield some nineteen or twenty compounds known as amino-acids. In the protein molecule the amino-acids are linked together to produce the mosaic of the various proteins somewhat as the twenty-six letters of the alphabet are grouped giving various words. The so-called simple proteins on digestion yield only amino-acids and their

derivatives, whereas the complex proteins yield not only amino-acids but also other compounds. Microörganisms build up and break down both simple and complex proteins. It is the activities of the microbes which render the nitrogen of the proteins available to higher plants. Bacteria are therefore, a vital link in the nitrogen cycle.

Outline of Nitrogen Cycle.—Nitrogen occurs in plants and animals primarily in the form of protein. The plant protein may be eaten by the animal, and there results animal protein. Either may reach the soil and decay. The nitrogen eaten by animals may be deposited as tissues of the animal or excreted as urea,

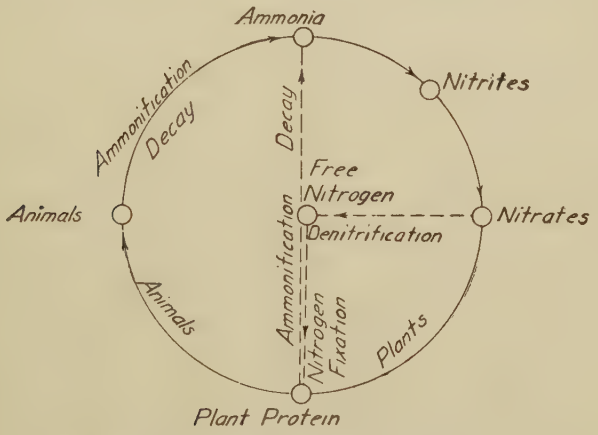


FIG. 43.—Illustrating nitrogen cycle in nature.

hippuric, or uric acid. These products are decomposed by microörganisms with the formation of ammonia.

Either the plant or animal proteins may reach the soil where they decay with the formation of the simpler compounds—albumoses, proteoses, peptones, peptids, and amino-acids. The amino-acids are then deaminized with the production of an acid and ammonia. The process is spoken of as ammonification.

The ammonia does not accumulate in the soil but is acted upon by other bacteria, the nitrosomonas, with the formation of nitrous acid. This is quickly taken up by the nitrobacter and oxidized to nitric acid which reacts with bases in the soil with the formation of nitrates. The nitrates are the main source of nitrogen for the plants which with nitrates and carbon dioxide build amino-

acids, peptids, peptones, proteoses, albumoses, and finally plant proteins—and the nitrogen has completed its cycle.

At different stages in this cycle chemical or biological agencies may liberate elementary nitrogen. It is then inert and useless to animals and most plants and can be made to reenter the nitrogen cycle again only by the addition of energy.

Portals of Entry into Cycle.—Nitrogen appears to have been the leftover after the completion of the earth, and as such occurs primarily in the inert atmospheric form. Thus, we ask: How did it enter the cycle, and when once removed, how can it again return? Each flash of lightning in the billions of storms which have occurred since the founding of the world has caused it to combine. Every volcanic eruption has thrown it into the atmosphere to be washed into the soil by the succeeding storm. The magnitude of this may be seen from calculations made by Arrhenius. He finds that the quantity brought to the earth's surface each year amounts to the stupendous quantity of 1,500 million tons. This is scattered with indifference on water, stones, and soil; and the quantity reaching each acre is small, and its concentration by man is an impossibility. Such a task would be somewhat similar to the extraction of gold from sea water. It has been estimated that if all the gold in sea water were extracted and apportioned evenly to all the inhabitants of the globe we would each be millionaires three times over. But it would cost more to recover it than the value of the gold.

Some years ago Hellriegel and Welfarth discovered that legumes when associated with certain bacteria possess the power of using atmospheric nitrogen. This is built into proteins and on decay becomes available to all plants. Still later Beijerinck discovered large yeast-like microörganisms which live free in the soil and take from the atmosphere nitrogen and build it into complex compounds. Every year since this date the number of organisms found to possess this valuable property has been added to, until today we know that in a measure it is possessed by most microörganisms. Very recently Lipman has shown that even some non-legumes may to a limited extent assimilate atmospheric nitrogen. True, each plant or microörganism fixes but a minute quantity and the quantity gathered on an acre yearly is not large, but given time and nature's process of concentrating, it becomes considerable, as is exemplified by the deposits in South America and the excessive concentration found in some western soils.

Within recent years the chemist has learned to substitute the electric arc or the electric current for lightning in order to capture atmospheric nitrogen. In the Birkeland and Eyde process air passed through an electric arc is fanned into a broad disk by a magnetic field, and from this there issues oxides of nitrogen. In the cyanamide process a strong electric current is passed through a mixture of lime and coke. The result is calcium carbide. When a stream of nitrogen is passed over this white hot compound lime nitrogen results. This can be used directly,

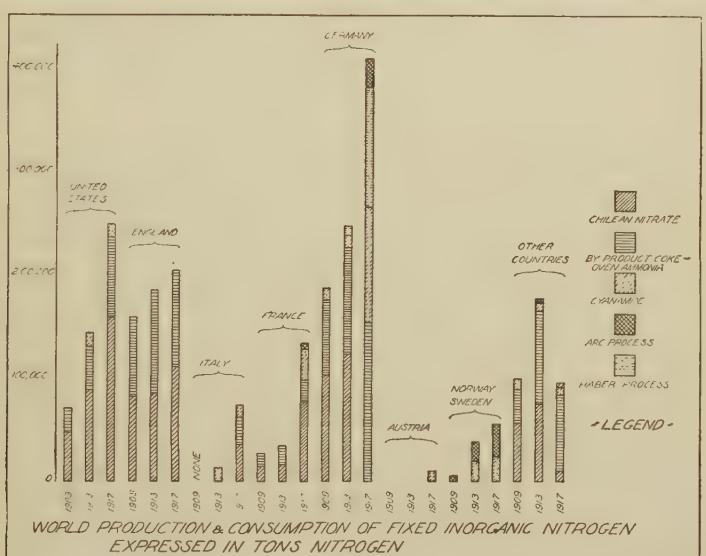


FIG. 44.—Illustrating production and consumption of fixed inorganic nitrogen expressed in tons.

or change to ammonia by treating with superheated steam. Today in countries where the required energy can be obtained from either natural or artificial waterfalls large quantities of nitrogen is being fixed and is successfully competing with the natural product. Where water power is not available, nitrogen is also being captured but by a different process. A mixture of hydrogen and nitrogen is passed over uranium, osmium, or platinum. These act as catalysts causing the hydrogen and nitrogen to combine directly with the production of ammonia.

Leaks in the Nitrogen Cycle.—There are few gates through which nitrogen may enter the cycle but many by which it may

leave. It is liberated when the bodies of plants and animals are burned. Millions of pounds of it reach the sewers, and from here is carried to rivers, lakes, and oceans. The compounds containing it are broken down, and the nitrogen is transformed into ammonia, nitrites, or nitrates. Various microörganisms liberate gaseous nitrogen from these. Every discharge of a firearm liberates it. Modern explosives are each year returning millions of pounds to the atmosphere. In some of the battles of the world war enough nitrogen was liberated to produce the food of the civilized world for a year. Its energy is being used in the digging of our canals, in the making of our tunnels, in the construction of our railroads, and in the mining of our coal. In each case it leaves the cycle only to return when outside energy is supplied.

Ammonification.—The soil is a large digestive system in which plant and animal residues are worked over so they may again serve as food for higher plants. The digestion is due to microörganisms which decompose the organic compounds so that they may obtain energy and building material. Incidentally, some of these decomposition products serve as food for higher plants. This is especially true of the nitrogen-carrying compounds. Microörganisms split proteins, and proteoses result. Proteoses are decomposed with the production of peptones; peptones yield amino-acids. These in turn are either oxidized or hydrolyzed, forming ammonia, and nitrogen-free compounds. These latter products are used by the microörganisms as a source of energy. Small quantities of the nitrogen have been used in the building of their body proteins, but by far the greater quantity of it is left in the soil to be changed into nitrites and finally nitrates by other microörganisms. Hence, we speak of the decomposition of nitrogenous substances by biological agencies with the production of ammonia, as ammonification.

Materials Ammonified.—Although all nitrogenous substances can be decomposed by microörganisms and usually with the formation of ammonia, the speed with which this transformation occurs varies with the compound. Urea and the simple proteins (especially those found in fish and blood) are rapidly decomposed by many bacteria. The complex proteins found in cow manure and cotton-seed meal are more slowly decomposed, whereas those in leather and bone are quite resistant. Some fungi (but not bacteria) are known to be able to decompose the

commercial nitrogenous fertilizer, cyanamide, with the production of urea. This latter product is readily transformed into ammonia by various bacteria.

The speed with which compounds are decomposed varies greatly, but ultimately all organic nitrogen is ammonified. Even such resistant or poisonous substances as chitin, quinine, strychnine, morphine, nicotine, and even the toxins produced by pathogenic bacteria are not exceptions. The rapidity with which various materials are ammonified is illustrated by the following results reported by Lipman and his associates: (The numbers represent the percentages of the total nitrogen of the various substances which were transformed into ammonia in six days.)

Concentrated tankage	56.66 per cent
Ground fish	47.16 per cent
Cow manure (solid and liquid)	32.60 per cent
Dried blood	16.74 per cent
Bone meal	16.65 per cent
Cow manure (solid only)	5.39 per cent
Cotton-seed meal	4.95 per cent

The nitrogen of straw is slowly ammonified. This is due to the nature of the nitrogen compounds occurring in the straw and also to the carbohydrates present, thus, making it unnecessary for the microörganisms to split the proteinaceous products. The general rule seems to be as follows: The addition of a carbohydrate to a medium delays the decomposition of the nitrogenous materials. Hence, when carbohydrates are added to a soil rich in straw or other plant residue they reduce for the time being the speed with which ammonia is produced. This may actually manifest itself by a decrease in the crop yield. It might thus be said that the speed with which ammonia is produced in a soil varies with the quantity and kind of manure applied. It also varies with the soil, for a rich sandy soil supplied with optimum moisture may ammonify many times faster than a depleted soil, or even a rich, tight, clay soil.

The optimum moisture content (if stated as percentage) of water in a soil varies greatly in different soils. For a tight clay it is very high and for a sand very low. However, if the water content of the soil is expressed in terms of its water-holding capacity it has been found that ammonification is at its maximum when the soil contains 60 per cent of its total water-holding

capacity. The ammonia produced in a soil increases as the water content increases until this limit is reached. Above this there is a sharp drop in the ammonia produced. The reason for this can be readily understood when it is recalled that in the last stage of ammonia production oxygen enters and ammonia is split off, consequently, aëration increases ammonification. It is rapid in fallow and hoed soils in which potatoes, corn, and beets are planted but it is slow in oats, wheat, and alfalfa soil.

The ammonifying organisms grow best in a neutral or slightly alkaline medium. Acid soils ammonify slowly, and probably the small quantity produced is due primarily to molds. Either lime or limestone added to such a soil accelerates the process.

Microörganisms decompose nitrogenous substances primarily to obtain energy for the carrying-on of their life processes. They must also have certain elements to be used in building their bodies. This is especially true of phosphorus which is needed in large quantities. Therefore, a soil having a goodly supply of this element in an available form liberates larger quantities of ammonia than a soil poor in phosphorus, provided other conditions are ideal.

Organisms Causing Ammonification.—Although it had been known for a long time that small quantities of ammonia occur in all arable soils, its formation was not known to be due to bacteria until 1893, when Müntz and Condon demonstrated that ammonia is not produced in sterile soils. Following this, they and other workers tested many bacteria, yeasts, and molds and found that numerous microörganisms possessed the power of producing ammonia. Even many

Fig. 45.—B. mycoides. (*After Conn.*)

Fig. 46.—Ps. fluorescens. (*After de Rossi.*)

of the pathogens when grown under appropriate conditions produce ammonia. *B. mycoides* and related sporebearing organisms were found to be very active ammonifiers under laboratory conditions; hence, it was quite generally accepted that they played a very prominent part in the production of ammonia in soil. However, the work of Conn makes this appear very doubtful,

for he found that these organisms, while in the soil, exist primarily as spores, and were thus inactive. But, he did find that *Ps. fluorescens* and related non-spore-forming organisms take part in the production of ammonia in manure and manured soils.

Numerous soil fungi have been studied which rapidly produce ammonia, and it is the opinion of many soil workers at the present time that these take a more prominent part in ammonification in soil than do bacteria. Probably there is a variation in different soils, depending upon the proteinaceous substance present. One organism may act best on one substance and in one soil, whereas another organism may be at its maximum efficiency in an entirely different soil working on other material. As these organisms exist in soil probably there is a division of labor. Some rapidly split the complex proteins; others act on the albumoses; and still others act on the amino-acids. There are bacteria which quickly break down urea with the production of ammonia. This may often be detected by its odor in a stable on a warm day. Although we find a great variation in activity and substance on which the ammonifiers act, yet they are all working in keeping with the general scheme of things—the working over of old discarded material and rendering it suitable again to be used by plants and eventually by animals. The numbers in a soil vary, but probably from 10 to 25 per cent of the soil flora are active ammonifiers.

Importance of Process.—We have seen that ammonification is a process by which complex compounds are changed into simple ones. It is obvious that higher plants cannot feed on fish, meat, or manure, but when these are changed into nitrates they become excellent sources of plant-food. The process of ammonification then is one step in the production of available plant-food. Therefore, anything which will accelerate it during the growth of the plant is beneficial, whereas anything which retards it is detrimental.

Moreover, anything which would accelerate it during the wet seasons when the plants are not on the soil is injurious, because as the nitrogen changes into ammonia (and especially when transformed into nitrates) it is soluble and can be leached from the soil. The quantity of ammonia in a good arable soil is about four pounds per acre-foot. Occasionally ten times this amount may be found in pasture or heavily manured soil. In special cases even larger quantities may occur, but then abnormal plant growth occurs.

Nitrification.—Ammonia does not accumulate in the soil, but as rapidly as it is produced it is transformed into nitrites and then into nitrates—a process known as nitrification. Although it has been known for a long time that nitrates are produced in soil (and Pasteur even suggested that it was a biological process) the first experimental report in this connection appeared in 1877. This was furnished by two chemists, Schlösing and Müntz. They tried to ascertain whether or not humus is essential in the purification of sewage by soil. Glass tubes one meter in length were filled with ignited sand and powdered limestone. At first the sewage passed through this filter unchanged. Later nitrates appeared, and soon the filtrate contained instead of ammonium salts an equivalent quantity of nitrate. Microörganisms were suspected of being the active agents; hence, the filters were treated with chloroform. Nitrification ceased, and the ammonia in the sewage passed through unchanged. A water extract of fresh garden soil added to the tube soon restored nitrification.

Warington at once applied this new discovery to soil processes. He showed that nitrification in the soil is stopped by chloroform and carbon bisulfide and that solutions of ammonium salt can be nitrified by adding a trace of soil. By long and careful experiments he showed that the process occurs in two stages, the ammonia being first converted into nitrites, and then into nitrates. Even after years of study he failed to obtain pure cultures of the organisms.

Pure cultures of the nitrifying bacteria were first obtained by the Russian investigator, Winogradsky, in 1890. He showed that these organisms are sensitive to organic matter and, hence, did not develop on the gelatin plates which were used at the time in the study of soil organisms. He found the following quantities of organic matter to inhibit the growth and action of the nitrifying organisms in alkaline solutions:

	Peptone, per cent	Asparagine, per cent	Urea, per cent	Glucose, per cent	Ammonia, per cent
Nitrosomonas....	0.2	0.3	?	0.2	
Nitrobacter.....	1.25	0.5 to 1.0	1	0.2 to 1.03	0.015

He grew them for some time in media containing only mineral salts, after which they were transferred to gelatin plates. A few

colonies developed, but having learned that none of these were the organisms sought, he concluded that they might be at the points where inoculations had been made but no growth was visible. He then, inoculated media with material picked from these points and in this way obtained the organisms in pure cultures. Successful platings were made only after Kühn introduced silica jelly for such purposes. Later it was shown by Beijerinck that agar can also be used after it is carefully washed free from all soluble material. Winogradsky found the nitrite producers to grow either as small oval, motile rods with polar flagella, or in larger non-motile globular forms. He named them *nitrosomonas* and *nitrococcus*, respectively. Both possess the power of transforming ammonia

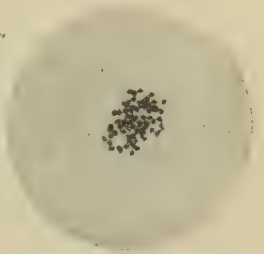


FIG. 47.—Nitrifying bacteria nitrococcus. (*After Bonazzi.*)

FIG. 48.—Nitrifying bacteria nitrobacter. (*After Gibbs.*)

into nitrites. The organisms which oxidize the nitrites to nitrates he designated *nitrobacter*. These are small non-motile

rods and transform the nitrites into nitrates as fast as it is produced in the soil, and it is only under abnormal conditions that either ammonia or nitrites accumulate.

In fact the speed of the reaction is governed by the rapidity with which ammonia is produced in the soil. Claims have been made that there are individual organisms in the soil that can bring about the two processes. Recently Sack reported that he had isolated from the soils of Holland a non-motile, gram-negative organism, *Nitrosomonas groningensis* capable of oxidizing ammonium salts to nitrites and to nitrates. He found that the organism thrives best in the presence of a large amount of organic matter; that it attacks cellulose and liquefies gelatin; and that, in the absence of oxygen, it reduces nitrates to nitrites and forms ammonia from proteins. If later work confirms these findings, it will revolutionize our ideas concerning the specialized activity of nitrifying organisms.

Distribution of Nitrifers.—Probably the nitrifying bacteria were some of the first living organisms on this earth. Even to-day they are the pioneers who prepare the soil for other plant life. Müntz has found them on the rocks of alpine summits where no other life exists. The organisms obtain their carbon from the atmosphere and their energy from the nitrogen compounds brought to them by the snow and rain. The ammonia is converted into nitric acid. This together with the carbon dioxide of the air, renders available the essential elements contained within the rocks.

They have been found in all normal cultivated soils and even in the sands of the desert. In humid regions they are confined principally to eighteen inches of the surface, but in districts where the soils are well aërated they have been found at a depth of five and six feet. They are not usually found in the air or in rain water, but river water and sewage contain them, and they are usually present in well waters. In the case of deep wells their origin is probably due to surface drainage which has found its way into the well, as the water of deep wells is not their natural habitat. Thomasen found the nitrosomonas in samples of ooze from the bottom of the Fiel Fiord, but not in the sea water, nor on the Plankton, or the fixed algae. They were also found in similar samples of soil from the vicinity of Helgoland and in slime from the bottom of the Bay of Naples, but only in samples taken near the land. They are not found, nor do they function, in sea water.

Factors Governing Nitrification.—Winogradsky concluded from early work that all soluble organic substances must be decomposed before nitrification can take place, and all ammonia must first be converted into nitrites before nitrobacter can commence work. It is self-evident that this cannot be rigidly applied to soil, as soils which have received large quantities of barnyard manure rapidly nitrify. Moreover, it has been shown that the soil humus is not detrimental but usually very favorable to the nitrifying organisms in soil. These apparent differences come from the fact that laboratory tests were made in solutions, the aëration of which was poor. In the soil it is good. Furthermore, the quantity of soluble organic material in soil is usually below that which Winogradsky found to be injurious. Ammonia exists in the combination of salts which are not injurious to the nitrifiers. In peaty soil and even in other soils where there is an acid reaction or a strongly alkaline reaction nitrification does not occur but can usually be started by neutralizing the acid or removing the excessive quantity of soluble salts.

The nitrifying bacteria, although light-avoiding are heat-loving, as illustrated by the following results: King found that there was produced during a year in an acre-foot of soil 120 pounds of nitrates at 1° C.; 150 pounds at 9° C.; 329 pounds at 20° C.; and at 35° C. there was produced 747 pounds.

They are also dependent upon a suitable moisture content of the medium in which they are growing. When only small quantities of water are present in a soil nitrification is slow. If too great an amount is present the nitrates disappear. The percentage quantity necessarily varies with different soils. However, the senior author has shown that if the water-holding capacity of the soil be determined and 60 per cent of this quantity be added to the soil, nitrification is at its maximum insofar as water is concerned.

The nitrifying bacteria are similar to other plants in that they can live on inorganic salts. Even carbon dioxide suffices as their source of carbon. The green plants with few exceptions are the only living organisms known to use carbon dioxide. These get the energy necessary to do this through the action of their chlorophyll on sunlight. The fuel which the nitrifying ferments burn in their tiny engines is ammonia in the case of nitrosomonas and nitrites in the case of the nitrobacter. The ashes from this quiet but interesting combustion are nitrates. Workers have traced a definite relationship between the amount

of ammonia oxidized and the carbon assimilated, as may be seen from the following:

	Experiment			
	1	2	3	4
	Mgm.	Mgm.	Mgm.	Mgm.
Ammonia oxidized (expressed as nitrogen)	722.0	506.1	928.3	815.4
Carbon assimilated	19.7	15.2	26.4	22.4
Ratio—nitrogen: carbon	36.6	33.3	35.2	36.4

Hence, thirty or forty pounds of nitrogen are oxidized in soil by these organisms for every pound of carbon they assimilate.

There is also a definite relationship between the ammonia oxidized and the oxygen consumed. Bonazzi has found this to be one atom of nitrogen to three atoms of oxygen for the nitrosomonas. He further found that a rapid nitrification occurs even in solutions if they are aërated by slowly shaking the container in which they are growing. It has been found that stirring or pulverizing a soil greatly increases its nitrate content.

Function of the Nitrifiers.—The nitrogen used by most plants is in the form of nitrates; so the main function of the nitrifiers is to render this element available to the higher plants. The acids produced have an indirect effect in rendering available phosphorus and potassium. However, the soluble nitrates are readily washed from the soil, and in regions where the annual rainfall is large or when excessive quantities of irrigation water are applied to a soil the loss in the drain water may even exceed that taken up by the growing plant. This, the careful farmer tries to prevent by growing crops on his soil while nitrification is rapid.

Nitrate Transformation.—Under normal conditions nitrates do not accumulate in the soil but are removed nearly as fast as produced. This removal takes place in the following manner: (1) They are taken up by the growing plants and transformed into proteinaceous material. This is a legitimate loss, and, if conditions could be made ideal, would be the main way by which they disappear. It is evident that its magnitude depends upon

the size and kind of crops grown. (2) Large or small quantities of nitrates may be washed from the soil, depending upon the nature of the soil, the plants growing upon it, and the volume of water which reaches it. In some deep arid soils this may be zero, whereas in shallow well-drained humid or heavily irrigated soil the quantity carried from the soil by this method may exceed that taken up by the crop. It is a dead loss and every means should be taken to prevent it. (3) The nitrates may be assimilated by microörganisms and built into bacterial proteins. Later, these may be nitrified and again rendered available to higher plants. This process is most rapid in soils containing decaying organic material and under some conditions may become great enough so that the microörganisms compete with the higher plants for the limited supply of nitrates in the soil. This is exemplified when straw or fresh manure is plowed under a few days or weeks before a new crop is planted. Under these conditions the straw or manure may have a distinct retarding effect upon the crop, because the carbon compounds will permit the soil microflora to assimilate the soluble nitrogen compounds previously formed in the soil. For a similar reason manures containing large quantities of organic material have a more lasting influence upon the productivity of the soil than do commercial fertilizers. (4) The nitrates may be partially reduced with the production of nitrites or even ammonia. Many organisms exist in the soil which have the power partially or completely to bring about these reactions. The reduction of nitrates to nitrites may occur under aërobic conditions, but the transformation of the nitrites to ammonium is confined almost entirely to anaërobic condition. Thus, it occurs only in peaty, swampy or water-logged soil. Neither process is of any economic importance in the soil as the nitrites or ammonia produced are reoxidized to nitrates as soon as aerobic conditions are established. The process is at times made use of in cheese-making. Nitrates are added to the curd and are decomposed by the bacteria present, thus preventing them from decomposing the lactose resulting in the formation of hydrogen and carbon dioxide which may cause ill-formed cheese. (5) The nitrates may be completely reduced, the nitrogen escaping as free nitrogen. This process is known as dentrification, and when it occurs in soil is of economic importance, as the nitrates are permanently lost.

A rather large number of dentrifying bacteria have been described and often named *Bacterium* or *Bacillus denitrificans*

with Roman numerals to designate the specific variety. *Bacterium stutzeri* is a common variety, and *fluorescens*, *putida*, and *radiobacter* all possess the power. However, the reduction occurs only under appropriate conditions, such as in the presence of nitrates; and suitable organic material; and in the absence of free oxygen.

Factors Influencing Denitrification.—Denitrification can take place in a soil only when a source of carbon is present, nor is the form in which the carbon exists without significance. Denitrifiers need large quantities of organic food and develop best in the presence of the fresh plant and animal debris. From the standpoint of production it would thus be unwise for the farmer to use heavy dressings of nitrate and fresh manure, or to plow under with the nitrates green-manuring crops. Partly decayed barnyard or green manures do not appear to favor denitrification.

In very wet soils oxygen is excluded. Here the bacteria turn to the soil nitrates for oxygen and liberate the nitrogen. In well-aërated soils this does not occur as the denitrifiers appear to be only facultative anaërobes.

The senior author failed to find any evidence of denitrification in a highly calcareous soil to which had been added from zero to twenty-five tons per acre of manure and from 12.5 per cent to 22.5 per cent of moisture, as may be seen from the following:

Treatment	Nitric nitrogen (per cent)	Gain in total nitrogen (per cent)
12.5 per cent of water*	100	100
15.0 per cent of water	118	108
17.5 per cent of water	121	102
20.0 per cent of water	121	104
22.5 per cent of water	123	108

* The soil containing 12.5 per cent of water was taken as producing 100 per cent.

Therefore, denitrification may occur in a water-logged soil containing large quantities of fresh organic matter or in tightly packed soil, but it is doubtful if it is of any economic importance in normal soils.

Denitrifiers are mostly heat-loving microbes, and hence have an optimum temperature relationship considerably higher than that which occurs in the average soil. The conditions, therefore,

which promote denitrification are just the opposite of those which promote nitrification. It is plain that the factors which the farmer has learned promote plant growth retard the growth of injurious bacteria.

Functions of Denitrifiers.—When acting in the farmer's soil, the denitrifier is a robber bacteria lying in wait until the banquet has been prepared by the honest-toiling nitrifiers. Then the denitrifier slips in and wastes the precious plant-food. But from what has been said concerning its habits, we find this microbe a robber by force of circumstance imposed upon him by the farmer's injudicious handling of his soil. However the denitrifier has a specific function in returning to the atmosphere the huge quantities of nitrogen which find their way in the form of organic compounds into the septic tanks of large cities. Moreover, thousands of tons of organic nitrogen are carried by streams to lakes and oceans. The nitrogen of this must be returned to the atmosphere so as to start again on its wonderful journey of construction, or in the case of war, destruction. How shall this be accomplished other than through the action of the denitrifiers?

CHAPTER XV

NITROGEN FIXATION, NON-SYMBIOTIC

Plants require ten elements for their normal growth and fruition. Three of these (nitrogen, phosphorus and potassium) are in soil only in small amounts as compared to the quantity required by the growing plants. One, nitrogen, is the limiting factor of crop production in most soils. The ancients, without a knowledge of these fundamental principles, supplied these essential elements to the soil in the form of manures, animal refuse, bones, and fish. Even the Indians taught the early settlers of this country to drop a fish into each hill of corn they planted. And ever since the discovery of the nitrate beds of South America this product has been used on the soils of Europe, Asia, and America. Although many new sources of combined nitrogen had been discovered and utilized, yet William Crookes, the discoverer of the Crookes tube, startled the world in 1898 by declaring that it was nearing the limit of wheat production and that by 1931 the bread eaters would have to turn to other grains or restrict their population, whereas the rice and millet eaters of Asia would continue to increase. He pointed out that the quantity of available combined nitrogen was going to be a limiting factor unless means be obtained for the utilizing of the atmospheric nitrogen.

From that date on many careful students have sounded the warning that the population is rapidly reaching the saturation point and must be met by a restriction of the population or an increased production of food-stuffs. New arable land is fast diminishing, and in the very near future more food can be produced only by the removal of the limiting factor of production. This at the present time, on the majority of soils, is the available nitrogen. Nitrogen fixation has consequently become one of the most vital problems which confront not only the tiller of the soil but mankind in general. This general conclusion has been reached by all who have given the subject careful thought, and as a consequence nitrogen fixation processes are and have been for a number of years the subject of intensive investigations

and development in many countries. This is especially true in Germany, France, Italy, England, Norway, Japan and the United States.

Nitrogen fixation on a commercial scale was first accomplished some twenty years ago, and at the present time, air-fixation processes produce annually the equivalent of three and one half million tons of Chilean nitrate. During the World War the Germans developed the synthetic processes to such a degree that they became independent of the natural occurring supply. This promises to be the case also with other nations in the near future. At present the requisite nitrogen can be fixed both for the arts of peace and war. However, such nitrogen is expensive, and it will be some time before it can compete with nature's method of fixing it in each acre of agricultural soil. It is essential, therefore, to learn the methods and conditions under which the microbe fixes nitrogen for man.

Development of Biological Idea.—The accumulated experience of ages caused man to conclude long ago that uncropped soils increase in fertility. More recent is the knowledge that the improvement is due to a gain in nitrogen. Why soils gain nitrogen has been known for only a short time. For decades it was attributed to chemical factors. Various inanimate constituents of the soil were supposed to act as catalyzers and cause the nitrogen of the air to combine with oxygen in the soil. The ideas of fermentation were fast being developed under the guidance of that brilliant Frenchman, Pasteur during the middle of the nineteenth century and his discoveries were applied in many fields. About this time we find Boussingault writing "Vegetable earth contains living organisms—germs—the vitality of which is suspended by drying and reëstablished under favorable conditions as to moisture and temperature." He actually proved by exposing moist soil in shallow dishes that they gain in nitrogen, and he suggested that the gain was probably due to microörganisms.

Berthelot during the last quarter of the nineteenth century, made a careful study of the nitrogen transformations in soil. Soil with a known nitrogen content was kept bare for seven months and after due allowance was made for the combined nitrogen brought to it in rain water he found that gains in nitrogen of 10 to 46 per cent had occurred. Much of this he attributed to the action of microörganisms. He continued his work and endeavored to isolate specific organisms which

possessed nitrogen-fixing properties. He proved that there exists within the soil chlorophyll-free microörganisms capable of fixing atmospheric nitrogen, and that they function best at summer temperatures, between 50° and 104° F. Heating the soil to 230° F. was found to stop the process. He further showed that these organisms are aërobic and function best in soil with a moisture content of from 12 to 15 per cent. Carbon, in sugar, and small initial combined nitrogen content was found to be essential. The fixed nitrogen was insoluble in water; hence, he concluded that it was proteinaceous. He considered that the quantity fixed by microörganisms was limited, as the tests studied by him drew from the atmosphere only so long as the quantity in the medium was not great.

Isolation of Nitrogen Fixers.—Berthelot's results on the fixation of nitrogen by aërobic bacteria were published in 1893, and a few weeks later additional data were furnished by the Russian bacteriologist, Winogradsky. He employed a nutrient medium free from combined nitrogen but containing dextrose and mineral salts. Fifteen separate species of soil bacteria were isolated; only one, a long spore-bearing bacillus, grew well in nitrogen-free media and fixed appreciable quantities of nitrogen. The organism he christened *Clostridium pasteurianum*. Later Bredemann showed this organism to be a strain of the common anaërobic butyric acid bacillus (*B. amylobacter*) which can be isolated from all soil and under suitable conditions always fixes some nitrogen. Winogradsky found the organism fixed 2 mgm. of nitrogen per gram of sugar, while later tests have shown gains up to 6 mgm. per gram of sugar decomposed.

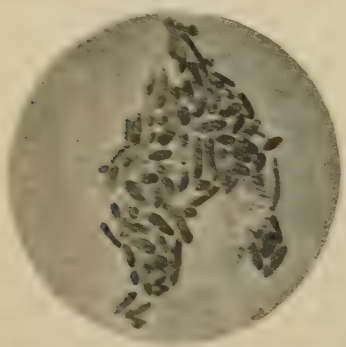


Fig. 49.—*Clostridium pasteurianum*. (After Winogradsky.)

A German investigator, Caron next used in vegetative experiments pure cultures of the bacteria most frequently encountered in natural soils. Some soils received bouillon cultures, whereas others received only sterile bouillon. The crop yields were usually in favor of the inoculated soil, but wide variations occurred from season to season. Exceptionally good results were obtained

with a spore-bearing bacillus which he termed *Bacillus ellenbachensis*.

This work led to the commercial exploitation of his cultures, one of which, "alinit," was the subject of much study and discussion. Later work showed alinit to contain two closely related bacilli which were designated *B. ellenbachensis* A and B. Both possessed the power of fixing nitrogen to a limited extent. However, later tests with "alinit" have failed to a great extent to confirm the claims of its exploiters.

In 1901 the Dutch bacteriologist, Beijerinck, described a large yeast-like aërobic organism which he named *Azotobacter*. These organisms in routine laboratory experiments frequently fix 10 to 15 mgm. of nitrogen for each gram of sugar assimilated, and under more favorable conditions even much higher gains have been reported. Since that time many bacteria, yeast, and molds have been shown to assimilate atmospheric nitrogen under appropriate conditions, and today it would appear that the power of fixing minute quantities of nitrogen is a characteristic of most microörganisms. Nevertheless, up to the present time the *Azotobacter* appears to be the champion nitrogen-gatherer.

Properties of Azotobacter.—Beijerinck described two species of Azotobacter—*Azotobacter chroococcum*, characterized by the production of chocolate-colored pigment, and *Azotobacter agile*, a rapidly motile organism, producing in solid and liquid media a brilliant green fluorescence. Later a number of strains of these organisms have been described. For instance, Lipman isolated from the soil of New Jersey *Azotobacter vinelandii* which Löhnis considers similar to *Azotobacter agile*. *Azotobacter beijerinckii* usually gives a white growth and in certain stages produces a distinctly yellow pigment. It is a strain of *Azotobacter chroococcum*. *Azotobacter vitreum*, which shows neither motility or pigmentation, is considered by Löhnis to be a strain of *Azotobacter agile*.

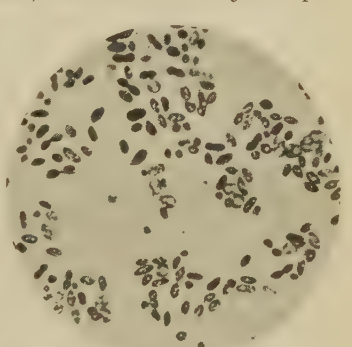


FIG. 50.—Azotobacter chroococcum. (*After Löhnis.*)

The organisms appear as short round rods 1 to 2μ in diameter and 1.5 to 3μ long. They have a great tendency to change

morphologically, the large yeast-like form often changing to small coccoid and rod-shaped types. They also pass over into an amorphous or "symplastic" stage. The organism in this form appears under the microscope either as an unstainable, or as a readily stainable mass without any easily distinguishable organization, and, if not discarded as dead, later gives rise to new vegetative forms.

Occurrence of Azotobacter.—Azotobacter are widely distributed, occurring in most normal soils. Lipman examined forty-six soils from various parts of the world and found *Azotobacter* in about one-third.

Several hundred Utah soils have been examined and found to fix nitrogen—many of them without the addition of carbohydrates. Nitrogen fixers occur more frequently and in greater numbers in cultivated than in virgin soils. This probably accounts for the much higher nitrogen-fixing power of cultivated soils.

Azotobacter were found in only two out of sixty-four localities in the soils of Danish forests. Both of the soils which contained them were from beechwood forests and contained calcium carbonate. Although the soils of these forests rarely contain enough carbonate to effervesce, they are usually neutral or slightly alkaline. They contain calcium, but in forms other than the carbonate. It is generally believed that *Azotobacter* occur commonly in soils which contain sufficient calcium carbonate to effervesce when acid is added and that they scarcely ever occur in acid soils.

Azotobacter are confined almost entirely to the first three feet of soil, although they have been found in soil at all depths down to the tenth foot in the very favorable constituted loess soils of Nebraska. They are most active in the upper few inches of soil, as is indicated by the following results obtained by Ashby:

Soil	Depth (cm.)	Average nitrogen fixed (mgm.)
Little Hoos	10	9.23
Little Hoos	20	7.29
Little Hoos	30	4.60

Reports on some Hawaiian soils show them to be equally active at all depths to four feet, but this must be considered an excep-

tion for the examination of numerous soils in Utah has shown a gradual decrease in nitrogen-fixing powers with depth. The average of several hundred determinations in both solution and soil media are given below:

Depth of sample	Nitrogen fixed in 100 gm. of soil with 1.5 gm. mannite	Nitrogen fixed in 100 cc. of Ashby's solution with 1.5 gm. mannite
	mgm.	mgm.
First foot	5.28	2.11
Second foot	2.42	0.77
Third foot	1.55	0.58

Condition for Growth.—The distribution and the physiological efficiency of the nitrogen-fixing organisms, especially of the *Azotobacter* species, are governed by the physical and chemical properties of the soil, foremost among which is its calcium or magnesium carbonate content. Some workers make use of this in obtaining pure cultures, for it is found that by picking out the crystals of the carbonate from the soil and seeding them into nitrogen-free media the likelihood of obtaining the organism is greatly increased. The addition of calcium carbonate to a soil often increases its nitrogen-fixing power, the extent depending on the lime requirements of the soil and on the fineness of the added limestone.

It has been suggested that the *Azotobacter* be used as an index of the lime requirements of a soil. Christensen and Larson examined over a hundred soils of known lime requirement. They determined the carbon dioxide set free by acids, the amount of calcium dissolved by an ammonium-chloride solution, the behavior of the soil toward litmus, and the growth of *Azotobacter* within the soil. The result of this test was that the biological test agreed with the known condition in 90 per cent of the cases, the ammonium chloride in 50 per cent, the litmus in 40 per cent, and the carbon dioxide failed oftenest in indicating the correct condition of the soil. Latter work indicates that they do not tolerate a Ph greater than 6.

Food Requirements.—*Azotobacter* probably require for their nutrition the same elements as do the higher plants: namely, carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, sulfur, calcium, magnesium, iron, and possibly aluminum and

manganese. These microörganisms obtain their carbon and hydrogen from organic compounds, preferably from carbohydrates. Oxygen is obtained from the atmosphere, the other elements from the soil.

A marked difference between *Azotobacter* and the higher plants is that the former possess the power of obtaining their nitrogen from the air. In the presence of combined nitrogen, however, they become lazy and obtain their supply from the soil. Small quantities of nitrogen stimulate the *Azotobacter*, whereas large quantities discourage nitrogen fixation since the organisms live on the nitrates. This may be due to a number of causes: (1) direct toxic action of the salt; (2) antagonism of other organisms which it favors; (3) the using up of the energy supply by these organisms; and, (4) the discouragement of fixation by the use of sodium nitrate. They must also have water and a suitable temperature, conditions that appear to be the same as for the nitrifiers.

From Whence Their Energy?—The nitrogen-fixing organisms differ widely from other plants in their energy requirements. This comes from their causing nitrogen to combine with other elements. Energy must be supplied. This is obtained through the decomposition of organic compounds, particularly one of the carbohydrates.

Normally the energy is obtained from the plant and animal residues brought to the soil and differ in value as may be seen from the following:

Material	Nitrogen fixed in 100 c.c. of solution after 3 weeks
	(mgm.)
Fresh straw	10.0
Fresh stable manure	9.8
Fresh peat	9.3
Green manure	8.0
Beijerinck's mannite solution	5.6

After humification these materials are even more readily assimilated and nitrogen fixation is greater than when the unhumified substance is used.

It is certain that humus can act as a source of energy and usually stimulates nitrogen fixers, but the extent is governed

largely by the composition of the humus and by the quantity of available combined nitrogen which is supplied with it to the organism. In addition to this, corn roots, corn stalks, oak leaves, lupine, alfalfa, maple leaves, and pine needles may all serve as a useful source of energy to the nitrogen-fixing organisms.

Crops vs. Fallow.—Heinze called attention to the fact that the fallowing of soil increased its nitrogen-fixing power. This may be due to better aëration, moisture, temperature, etc., and not to any depressing influence exerted directly by the plant. Most experiments which consider plant and bacterial activity may be interpreted in this light. Hiltner maintains that the non-symbiotic nitrogen-fixing bacteria are stimulated by the growing plant roots. There may be considerable truth in this for the higher plants rapidly remove from the solution the soluble nitrogen compounds; in which case, the nitrogen-fixers would be forced, either to compete with the higher plants for the soil nitrogen, or else make use of their ability to live upon the atmospheric nitrogen. It is certain that different cultural methods vary sufficiently with crops to influence profoundly a soil's nitrogen-assimilating properties, for the *Azotobacter* occur more widely distributed in cultivated than in virgin soil. The analyses of hundreds of samples of cultivated and virgin soils in Utah have in nearly every case shown the virgin soil to have a low nitrogen-fixing power as compared with the cultivated soil. This was the case even when the soil was incubated without carbohydrates and the nitrogen determined directly. The average results for many determinations were as follows:

Soil	Nitrogen fixed
	(mgm.)
Virgin soil	6.99
Cultivated	14.28
Wheat	11.83
Alfalfa	12.24
Fallow	22.81

It would be possible to fallow or crop soils so continuously that extremely small quantities of plant residues would be returned to the soil, under which conditions there would be a decrease in nitrogen fixation. It is just possible that the high fixation noted in the arid districts where wheat is grown continuously

may be due to the method in vogue of leaving the greater part of the straw on the soil. This would act as readily assimilable carbonaceous material for the *Azotobacter*. Fallowing not only increases the assimilable nitrogen but also the available phosphorus of the soil, a liberal supply of which causes the *Azotobacter* to utilize its energy more economically. The conditions of moisture and aëration are much more nearly ideal in a fallow soil than in a cropped soil.

Methods of Fixation.—How different are the ways of man and the microbe! Man fixes nitrogen by means of a gigantic arclight in a chimney through which a current of hot air is blown. The flaming disk has a diameter of seven feet and reaches a temperature in the neighborhood of 6300° F. The gas which is obtained when dissolved in water yields nitric acid. Or, in another method, air is cooled to −194° C., the nitrogen boiled off, mixed with hydrogen in the proportion of 1 to 3, heated to a temperature of 550° C., and then passed under great pressure over a catalyzer. There results ammonia. This, mixed with oxygen and passed through platinum gauze, yields nitric acid. Thus, in all synthetic processes great variation in temperature, and complicated, expensive apparatus are used.

When the microbe fixes nitrogen there is also a real conflagration in which plant residues act as the fuel and the bacterial body as the furnace. But how different are the two! The microbe is 90 per cent water. It works in the dark, damp, warm soil, and generates little heat and no light. We know its end products are not nitric acid, ammonia, or cyanamid but complex proteins. These are similar to those found in plants, and are composed of the same amino-acids. Some are soluble, others insoluble, all are broken down by bacteria and yield food for the higher plants. We do not know the first products produced in their formation nor the marvelous way in which the nitrogen is enticed to join hands with carbon, hydrogen, and oxygen. We can only surmise and hope that the future will unravel the mystery. But when unravelled, as it surely will be, the wonderful advancements of the past decade will seem insignificant beside it.

Soil Inoculation.—High hope was entertained that the nitrogen problem in agriculture had been solved when Caron announced that he had prepared a culture of bacteria which would enable non-leguminous plants to utilize free atmospheric nitrogen, provided certain precautions were observed. Many of the results which he reported from pot experiments were clearly in

favor of the inoculated plant. Stoklasa was one of the first to study in detail the commercial preparation "alinit" which was placed on the market as a result of Caron's work. His findings were fully as favorable as Caron's, but the work of others soon demonstrated that alinit, neither in the laboratory, nor in the field had the ability to fix nitrogen. When Beijerinck discovered the free-living aërobic nitrogen fixers, the hope that soil inoculation might be so perfected that it would be beneficial to crops was revived, and since that time many investigators have attempted to inoculate soil to increase its productivity, but usually with negative results. Some have made great claims for soil inoculation. They have found that soils, inoculated with *Azotobacter chroococcum* and adequately supplied with carbohydrates and lime, show an increase in the number of nitrogen-fixing organisms and also an increase in crop yields.

There may be a decrease in the crop during the first year when carbohydrates and *Azotobacter* are added to the soil with a marked increase in crop during the second and third years. Even then the soil may be left richer in nitrogen than it was at first.

EFFECT OF DEXTROSE AND SUCROSE ON THE PRODUCTIVENESS AND NITROGEN CONTENT OF THE SOIL

Carbohydrate added per 100 gm. of soil	Crops obtained				Total nitrogen removed in crop	Total nitrogen left in soil Spring 1906	Nitrogen as nitrates (p.p.m.)
	Oats, 1905		Sugar-beets, 1906				
	Dry matter	Yield of nitrogen	Dry matter	Yield of nitrogen			
					Gm.	Percentage	
None	100.0	100.0	100.0	100.0	0.5914	0.093	10
2 per cent dextrose	32.3	62.5	186.0	190.0	0.6814	0.105	17
2 per cent sucrose	33.3	58.7	179.0	195.0	0.6800	0.105	15
4 per cent sucrose	37.7	78.1	283.0	339.0	1.0092	0.119	37

It is often the case that the addition of starch to a soil retards plant growth during the first year. This injurious action may be due to the increased bacterial activity in the soil brought

about by the carbohydrates which injure the roots of the plant by withdrawing oxygen and by forming hydrogen sulfide in the deoxygenated atmosphere of the soil through the reduction of sulfates by the bacteria. Or, it may be due to the increased microflora feeding on the nitrates; or even to a lack of nitrate production because of the abundance of non-proteinaceous food, which leaves the plants without proper food and thus causes them to starve.

The effects produced by the carbohydrate applications also vary with the season. If applied in the spring when the soil temperature is low and when other bacteria are more active than *Azotobacter*, the other bacteria multiply rapidly and compete with the higher plants for the limited available plant-food. If, however, the carbohydrates are applied in the autumn directly after the removal of the crop when the soil is warm, the *Azotobacter* are active and the result is that sufficient nitrogen is fixed to produce an increased crop the following season.

Many workers have noted either no effect or even a detrimental influence when soils are treated with carbohydrates and then inoculated with *Azotobacter*. This may be due to any, or all of the following factors: (1) absence of a suitable environment, such as proper temperature, moisture, aëration, food, and alkalinity; (2) absence of a suitable host from which *Azotobacter* may obtain part of its carbon; and (3) injurious effects due to the decomposition products of the carbohydrate added.

There is considerable interest in the work of Bottomley who uses bacterized peat or humogen. The bacterizing process consists of three stages: (1) treatment of peat with a culture solution of the special "humating" bacteria and an incubation of it at constant temperature for a week or ten days during which period soluble humates are formed; (2) destruction of the humating bacteria by sterilization with live steam; and (3) treatment of this sterilized peat with mixed cultures of nitrogen-fixing organisms—*A. chroococcum* and *R. leguminosum*—and incubating it at 20° C. for a few days, after which it is ready for use.

Theoretically, there is much in this process which recommends it, for there is no abrupt change in environmental conditions for the organism that is added, as is the case when an organism from laboratory culture is added. Moreover, they are added in enormous quantities and with a source of carbon which is not far different from that found in the soil. Russell, however, after carefully reviewing all of the experimental evidence on the

subject, concludes: "There is no evidence that humogen possesses any special agricultural value. There is not the least indication that it is fifty times as effective as farmyard manure, to quote an oft-repeated statement, and there is nothing to show that it is any better than any other organic manure with the same nitrogen content." Furthermore, he concludes that there is no definite evidence that "bacterization" really adds to the value of the peat.

It is evident that soil inoculation in order to be successful must be accompanied by the rendering of the physical and chemical properties of the soil ideal for the growth of the specific organisms to be added. A few organisms placed in a new environment already containing millions of other organisms can never hope to gain the ascendancy over those naturally occurring in the soil, for the latter have been struggling for countless generations to adapt themselves to the environment, and only those which are fitted have survived. The problem becomes even more complicated when we recall the findings in which Lipman pointed out the bacterial flora of a soil is in many cases entirely changed by climatic conditions. It would appear that to ever make soil inoculation a success the chemical, physical, and even the biological condition must be made suitable for the growth of the specific organism added. Furthermore, strains of the organisms must be used which have been evolved under similar climatic conditions.

Soil Gains in Nitrogen.—It is well established that many microörganisms, either when grown alone or in combination with other microörganisms possess the power of fixing nitrogen. Many of these have been obtained in pure culture and their morphology and physiology carefully studied. The most favorable conditions for their maximum nitrogen fixation of pure cultures in liquid solutions have been accurately determined. Some of the conditions requisite for their activity in soils are known, but on this phase of the subject there are many gaps in our knowledge and much work must yet be done before we can state definitely the part they play in the economy of nature, and before we can say which are the very best methods for increasing their usefulness. Nevertheless, it is interesting to consider the results obtained by a few workers.

Berthelot's early laboratory experiments led him to believe that sands and clays may fix in a year from seventy-five to 100 pounds of nitrogen to the acre. In two exceptional instances he noted that sands gained 525 pounds and 980 pounds of nitrogen

an acre, but soils containing fairly large quantities of nitrogen never made rapid gains.

Krainskii thinks that even better results should be obtained in soils than in pure solutions, for in the former the nitrogen fixers grow in symbiosis with autotrophic organisms which produce organic compounds available to the *Azotobacter*. In soils the fixed nitrogen is rapidly removed by other plants; therefore, the slowing-up process which becomes perceptible so early in laboratory experiments should not occur.

In addition to an optimum temperature and moisture content of the soil, the *Azotobacter* are dependent upon a supply of carbon for energy, and inorganic nutrients for the building up of cell protoplasm. Unfortunately, it is too often the case that under natural conditions those soils which are deficient in nitrogen are also lacking in available carbon, and especially in phosphorus, both of which are so essential for rapid nitrogen fixation. Then too, there are the technical difficulties which the chemist encounters in determining the gain or loss of nitrogen which occurs in soils under natural conditions and which may be attributed to non-symbiotic nitrogen fixation.

There are, however, several cases in which the gain in nitrogen has been measured with a fair degree of accuracy. Lipman, in pot experiments carried on with a soil containing about 5,000 pounds of nitrogen per acre-foot of soil, found a gain of more than one-third this amount in two short seasons. Much of this must be attributed to non-symbiotic nitrogen fixation. To these soils had been applied solid and liquid manure which furnished to the organisms readily available supplies of energy and various necessary inorganic constituents. This fixation was not nearly so rapid where legumes were turned under as green manures. Koch found a gain of 0.019 per cent in soil nitrogen during two seasons which must be attributed to non-symbiotic nitrogen fixation. In addition to this there was a threefold gain in the nitrogen content of the crops—oats, buckwheat, and sugar-beets—which must also be attributed to the action of the *Azotobacter*. Hall noted an annual gain of 100 pounds of nitrogen on Broadbalk field at Rothamsted and twenty-five pounds on Grescroft field. He feels that much of this gain must be due to the action of non-symbiotic bacteria.

The senior author has obtained appreciable yearly gains of nitrogen in a typical dry farm soil kept in two gallon jars with optimum moisture. Plant residues were added and then

the total nitrogen determined. At the end of two years the total nitrogen was redetermined.

YEARLY GAIN NITROGEN

Treatment	Yearly gain nitrogen lbs. per acre foot soil	Treatment	Yearly gain nitrogen lbs. per acre foot soil
None	35	3 tons pea vines	33
1 ton alfalfa	161	4 tons pea vines	0
2 tons alfalfa	304	5 tons pea vines	0
3 tons alfalfa	123	1 ton straw	77
4 tons alfalfa	254	2 tons straw	74
5 tons alfalfa	43	3 tons straw	loss
1 ton pea vines	248	4 tons straw	loss
2 tons pea vines	36	5 tons straw	loss

These figures represent the quantities of nitrogen fixed by the organisms in 3,600,000 pound of surface soil during one year.

The analysis of a great number of soils in Utah showed that the average nitrogen content of the soil which had grown wheat and other non-leguminous plants for from twenty to fifty years was 0.2009 per cent, whereas adjoining virgin soil on the average showed only 0.1984 per cent of total nitrogen. The evidence is very strong that considerable nitrogen has been added to these soils by microscopic organisms, as is shown by the following:

(1) In nearly every case the cultivated soil fixed much more nitrogen in the laboratory than did the virgin soil. This was the case when the soil was incubated with or without the addition of carbonaceous material.

(2) There was a richer nitrogen-fixing bacterial flora in cultivated than in virgin soil.

(3) The conditions of moisture, alkalinity, and food constituents in the soil were ideal for rapid nitrogen fixation, and the temperature of the soil was high enough during a considerable part of the year for the growth of *Azotobacter*.

(4) The cultivation of the soil increased aëration and available phosphorus in the soil.

(5) The large quantity of plant residues received by the soils acted as a supply of carbon which was readily rendered available by the soil's rich flora of cellulose ferments. If these soils had produced a wheat crop every alternate year and all of the

nitrogen which had been added to the soil without loss from leaching or bacterial activity taken by the crop, it would have necessitated the addition of twenty-five pounds an acre yearly, which is evidently the very minimum which can be attributed in these soils to non-symbiotic nitrogen fixation.

Eighty different samples of these soils were incubated in the laboratory for twenty-one days and the gains in nitrogen determined by comparing with sterile checks. The soils were incubated without the addition of anything except sterile distilled water. At the end of the period the average gain per acre for the cultivated soils were 202 pounds and that for the virgin soil was ninety-two.

True, fixation would not continue long at this rate, for when the nitrogen content of the soil passes beyond a certain limit decay bacteria increase rapidly, and in the struggle for existence they are able with the advantages at their disposal to suppress the more slowly growing *Azotobacter* which would gain the ascendancy again only when the nitrogen of the soil became low.

Thus, there is an upper as well as a lower limit to the nitrogen content of the soil as far as bacterial activity is concerned, but by making the conditions for nitrogen fixation as nearly ideal as possible we may maintain in a soil the upper and not the lower nitrogen content.

In conclusion, it may be stated that although the part played by the *Azotobacter* in maintaining the nitrogen of the soil has not been definitely measured, it is nevertheless an important factor. Hall found gains of at least twenty-five pounds, Löhnis thirty-seven and seven-tenths pounds, and the senior author from twenty-five to thirty-five pounds per acre annually. The increase was much greater when suitable organic material was added. It is, therefore, conservative to state, with Lipman, that these organisms under favorable conditions add from fifteen to forty pounds of available nitrogen to each acre of soil yearly. If the non-symbiotic nitrogen-fixers were adding fifteen pounds per acre yearly to the 375 million acres planted to harvested crops this would represent an annual gain in nitrogen of 2.8 million tons. If by better cultural methods associated in some cases with inoculation the annual fixation can be raised to forty pounds per acre there would be a gain in nitrogen of approximately seven and one-half million tons.

CHAPTER XVI

NITROGEN FIXATION, SYMBIOTIC

The farmers of ancient Rome observed that crops following legumes were better than those following non-legumes. Even before the Christian era in the Far East legumes were used for green manure, and it has been the general experience of practical farmers throughout the ages that legumes increase the productivity of the soil. This knowledge has been crystalized in the slang phrase "too poor to raise beans!"

During the middle of the nineteenth century it was considered an established fact that plants could not use atmospheric nitrogen, yet practical experience and carefully conducted experiments pointed to the conclusion that the legumes possess peculiar powers. It is only within the memory of men now living that it has been learned that legumes are veritable nitrogen-fixing factories. It is to these that we must turn for our combined nitrogen, for in the words of Lipman: "At best nitrogen in commercial fertilizers can supply only a small part of the nitrogen requirements of crops. Energy costs may be reduced and superior catalysts may be developed in technical nitrogen fixation, but even then we shall have to depend on legumes, legume bacteria, and solar energy to give us combined nitrogen at the least cost. To that end we must not only seek to improve our farm practices as they apply to crop rotations, tillage, water supply, and the use of lime and mineral fertilizers, but we must search for more efficient types of legumes as well as more efficient types of bacteria, both symbiotic and non-symbiotic."

Early Views.—Van Helmont was one of the first to use the balance in studying plant growth. He planted a willow weighing five pounds in 200 pounds of soil. This he carefully tended for five years. At the end of that time he removed the soil and found that it had lost only two ounces. The willow had gained 159 pounds. He concluded that the willow had been produced from the water. He had overlooked the small quantity of carbon dioxide, which a century and a half later Priestley

decided came from animal life and was removed by plant life. What was more natural than for him to conclude that plants also obtain their nitrogen from the air? His conclusions rested on crude qualitative tests; hence, it remained for subsequent workers to fully establish the fact that plants obtain their carbon from the atmosphere.

Later Work.—Liebig considered all crops capable of feeding on atmospheric nitrogen, but the legumes and other broad-leaved plants he believed were especially fitted for this task (as witnessed by the fact that they benefit the succeeding cereal crop and do not respond as readily to nitrogenous fertilizers).

About 1834 that adventurous South American traveler, Boussingault, began a series of field experiments on his farm in Alsace. He studied no fewer than five rotations and kept records of the loss and gain of the various elements. Interesting enough, he found that when the rotations contained legumes there were gains in nitrogen.

Experiments conducted in closed vessels on heated soil, however, soon demonstrated that the free nitrogen of the air was not available to the higher plants. Could it be that they were feeding on the ammonia of the air? No, experiments showed that this was not sufficient. As a consequence it was accepted for a number of years as demonstrated that plants obtain their nitrogen only from the soil.

This was contrary to the agricultural experience of all ages, and the carefully conducted experiments of Rothamsted continued to point to the conclusion that legumes behave abnormally. Could it be that the deep-rooting legumes were bringing nitrogen to the surface?

Later work explained the apparent contradiction in showing that Boussingault had used sterilized soil, while plants normally grow in unsterilized soil. It is to Hellriegel and Wilfarth (1888) that credit is due for solving the mystery. They found that legumes grew quite normally in soils that lacked nitrogen, provided the soils were not previously sterilized. Growth was checked, however, in sterilized nitrogen-free soil because of the lack of nitrogen. The addition to the soil of a small quantity of an infusion from unsterilized soil produced normal growth of the plants and resulted in a crop rich in nitrogen. If the added infusion was previously boiled, its addition produced no effect at all; the plants were retarded in their development and the harvest showed no increase in nitrogen. It was found that the

soil used in preparing the infusion must be taken from a field in which plants similar to those used in the experiment had been growing. For example, if peas are used, the soil for the watery extract must come from a field where peas have previously grown. The legumes grown in unsterilized soil or in the inoculated soil had on their roots small knobs or tubercles. Those grown in sterilized soil did not possess these. This fact led to the conclusion that these tubercles played a part in the fixation of atmospheric nitrogen.

Prior to this Wigand (1887) found that the tubercles contained true bacteria, and the following year they were obtained in pure cultures by Beijerinck. He further found that there were bacteria associated with all tubercles, and although the bacteria differed somewhat in the tubercles of different species of plants, that there still were certain constant characteristics to be seen in them all. He, therefore, regarded the tubercles as the result of the action of bacteria and gave to the organism producing the tubercles the name of *Bacillus radicicola.* Since then it has been called *Pseudomonas radicicola* by those who consider it to have polar flagella. Others consider it sufficiently different from other bacteria to warrant making a separate genus and give to it the name *Rhizobium leguminosum.*

Beijerinck considered the bacteriods of previous workers as involution forms of the bacteria which appeared only after the bacteria have lost their vigor. In a later investigation, after isolating the bacteria and keeping them in pure cultures for many months, he was able to produce the tubercles at will by inoculating the soils in which his plants were grown with the pure cultures of the organisms.

Legumes.—It is the legumes which under appropriate conditions possess the valuable property of feeding on atmospheric nitrogen. They have certain characteristics by which they may be distinguished from the non-legumes: (1) They produce their seeds in a pod. (2) Their flowers are practically identical in the parts that form them. True, there is a variation in the shape and size of the parts as well as in the whole blossom, but a small flower picked from a head of red clover or a cluster of alfalfa blossoms shows the same characteristics. (3) Their leaves also show an interesting resemblance, each leaf being usually composed of leaflets arranged along a common stem. (4) Under normal conditions the legumes have on their roots round or club-like growths of various shapes and arrangements.

How the Nitrogen Is Fixed.—Only legumes which have on their roots nodules have the power of fixing nitrogen. These are small wart-like protuberances and are the homes of the nitrogen fixers. Nodules vary in shape and size according to the legume on which they occur and the soil in which they are found. Those found on the bean plant are usually round; those on the clover, oval; those on the soy-bean large and round; while those on the alfalfa are small club-shaped, and often grouped in finger-like bunches. They are large and numerous in well-aërated soil. In water-saturated or tight soil they occur near the surface and are sometimes colored similar to a sun-burned potato. While the plants are forming seeds most of the nodules are soft and the internal tissue sloughs off, leaving the more resistant outer tissues as a mere shell which later decays.

If one of the nodules is cut open and the inner part examined with a high-power microscope the symbiotic bacteria may be seen. These are minute rod-like organisms which may take on the form of stars, crosses and other grotesque shapes, depending upon the plant from which they come. Each nodule contains billions of these organisms. They get into the roots of the young, partly-starved plants. As the tip of the root hairs of the plant pushes itself out into the soil, it chances to come into intimate contact with the bacteria. They gain entrance, grow, and rapidly multiply, until finally a nodule is formed. They obtain their food from the plant juices. The plant gets its nitrogen from the bacteria growing in the nodules. Here we have two friends living together—each helping the other, each performing the task which it has become best suited to do through ages of specialization. The plant with its extensive specially constructed leaf surface drinks in the carbon dioxide and mysteriously gathers up the heat and light waves. These enter the cell (the laboratory of that master chemist, chlorophyll) where they are transformed into carbohydrates. This fuel is silently passed down to those tiny dynamos, the microörganisms, which fill the nodules on the root. Here the carbohydrates are burned, and the resulting energy is used to cause the atmospheric nitrogen to combine with hydrogen and oxygen. Some of the resulting product is used in the construction of more bacterial cells. Most of it is passed on to the plant and becomes a part of its tissue and later its seed. The bacteria probably receive their fuel in the form of a soluble carbohydrate, but we do not know the medium of exchange used in payment. It may be a nitrite, or nitrate, an

FIG. 51.—Root nodules caused by nitrogen fixing bacteria. A, Crimson clover nodules; B, alfalfa nodules; C, lima-bean nodules; D, cow-pea nodules; E, Canada-field pea nodules. (*After Kellermann.*)

amino-acid, or even a protein. We do know that it is a nitrogen-bearing substance which can be used by the plant in its constructive metabolism.

The Causative Organisms.—Beijerinck named the legume bacteria *Bacillus radicicola*, but since that time, as already mentioned, various generic names have been applied to the organisms. They are rods, and when full-grown vary in length from one to four or even five microns. It is not uncommon to find them from 0.5 to

FIG. 52.—Bacteriods from a very young nodule of pea. (*After Burrill and Hansen.*)

0.6 μ wide and from two to three μ long. Some have been found to measure only 0.18 μ in width and 0.9 μ in length. They have a great tendency to form involution forms, especially in the older nodules, appearing as stars, crosses and various grotesque forms. The organisms do not stain readily with the ordinary aniline stains. Carbol-fuschin and anilin-gentian-violet are the most satisfactory. The former brings out the vacuolated condition especially in the bacteriods. All nodule bacteria are motile, but the mode of flagellation varies. They do not grow well on the

ordinary laboratory media, nor do they readily fix nitrogen away from the host plant. Gains of from 2 to 3 mgm. of nitrogen per gram of mannite is about the maximum. However, they must be able to function to a limited extent in soil, for they have been found in soils years after the disappearance of the legume. It is quite possible that during this time they live in symbiosis with various simple plants which flourish in the soil.

FIG. 53.—Rhizobium leguminosum from nodule of cow-pea. (*After Burrill and Hansen.*)

Seven different varieties of legume organisms are recognized. These are classified according to the particular group of legumes that they invade:

1. Those which infect red clover, mammoth red, alsike, crimson, Egyptian, or white clover.
2. Those which infect alfalfa, sweet clover, bur clover, yellow trefoil, or fenugreek.
3. Those which infect the garden pea, hairy vetch, spring vetch, wild vetch, broad bean, lentil, sweet pea, or perennial pea.
4. Those which infect cowpeas, the partridge pea, peanut, Japanese clover, velvet bean, lima bean, wild indigo, or trick trefoil.
5. Those which infect the garden, navy, kidney or scarlet-runner bean.
6. Those which infect lupine, serradella, or wild lupine.
7. Those which infect the soy-bean.

Cross inoculation occurs from one plant to another within the same group, and infected soil or organisms may be successfully used within the groups.

Conditions Favoring Growth.—The legume bacteria are all aërobic and the nodules on the roots of the plants are usually near the surface. The nodules will also form on plants grown in water cultures, but they are not as large and active as when grown in a well-aërated soil. Moreover, the legumes obtain their nitrogen through the roots and not the leaves; hence, the influence of cultivation on legumes is threefold: (1) The loosening up of the soil brings atmospheric oxygen and nitrogen in contact with the nodule bacteria. (2) It increases the activity of decay bacteria which render essential plant food available to the higher plant. (3) The loose aërated surface tends to conserve soil moisture which can be drawn on as needed by the plant.

The root system of plants varies greatly with the moisture content of the soil. It has been found that legumes growing in moist soil have roots which spread widely, are high in water, are covered with root hairs, and present a large surface of young tissue—all conditions that are ideal for the growth of nodules large numbers of which are found on such plants. In dry soil the roots spread less and the epidermis is greatly thickened, consequently the tubercules are few and small on such plants.

The legume bacteria require the same elements for their growth as do other plants, and soils rich in available phosphorus and potassium, or fertilized with these constituents, produce numerous large active nodules if other conditions are ideal. Large quantities of available nitrogen, however, discourage the growth of the legume bacteria.

Most cultivated plants do best in an almost neutral soil reaction $P_H = 7$. This is also true for the legumes, and where there is either excess alkali or acidity in a soil it must be brought to the right reaction for maximum growth.

They do best in a neutral soil, yet they will tolerate some acid, the quantity varying with the different organisms. The extent to which the different legume bacteria will tolerate acidity may be seen from the following minimum P_H values obtained by Fred and Davenport:

Alfalfa group	5.0	Garden bean group	4.3
Pea and bean group	4.8	Soy bean group	3.4
Clover group	4.3	Lupine group	3.2

Influence of Nodules on Plant.—The nodules make it possible for the legume to utilize the atmospheric nitrogen. Soils poor in nitrogen but otherwise suited to the growth of legumes can

be made to produce excellent crops if properly inoculated, and much larger yields are often obtained from inoculated than from uninoculated legumes. Legume crops bearing nodules on their roots are richer in protein and, more valuable as a food than is a crop of the same legume which has borne no nodules. The seeds of the inoculated legume are usually superior to those of the uninoculated.

For centuries it has been the practice in China, Japan, Western Asia, and Northern Africa, to grow legumes and non-legumes in combinations, and there is no doubt but that time and again practical farmers have noted the more vigorous growth and

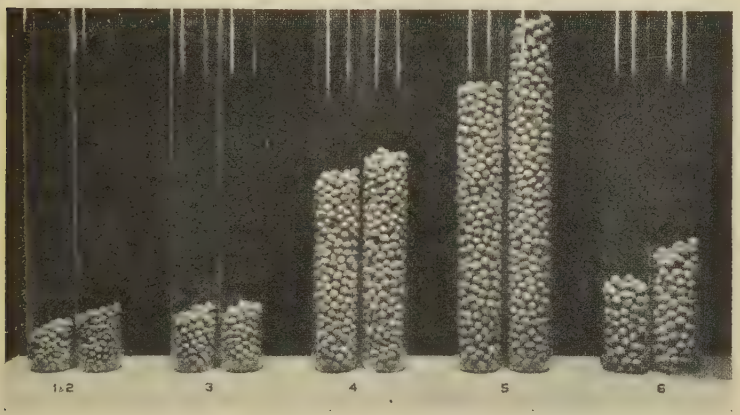


FIG. 54.—Influence of inoculations on yield of peas. (*After Fred, Whiting and Hastings.*)

darker green of non-legumes when so grown. The discovery of the ability of properly inoculated legumes to fix nitrogen made it seem likely that they were assisting the non-legume in obtaining atmospheric nitrogen. That this is actually the case was first demonstrated by Lipman. He grew non-leguminous plants in a porous pot surrounded by earth in a larger glazed earthenware pot in which leguminous plants were growing and found that under favorable conditions non-legumes associated with legumes may secure large quantities of nitrogen from the latter.

Inoculation of Legumes.—Legumes fix nitrogen only when properly inoculated, and many experiments have been conducted to learn how and when to inoculate. Some of the early attempts

failed because knowledge of the different strains of bacteria was lacking and improper methods of growing and distributing the bacteria were employed. These difficulties have been largely overcome, so that today reliable cultures are available from the government and various state experiment stations as well as commercial concerns. However, even these cultures have little or no value unless other conditions are ideal, as for example: (1) Good physical conditions of the soil. (2) The soils must contain the elements essential to plant growth, with the exception of nitrogen. (3) The soil must be a suitable home for the legume and bacteria, that is, it must have a correct reaction, moisture, temperature, and aëration if maximum results are to be obtained.

Fig. 55.—Bottle culture of root nodule bacteria. (*After Fred, Whiting and Hastings.*).

It has been found that in the majority of soils where other conditions are favorable the legume bacteria are present. This is especially true where a particular legume has been grown for some time. The organisms may have been in the virgin soil growing on some native legume, or carried to the soil by various mechanical means.

When a legume is being grown on a piece of land for the first time, or even where considerable time has elapsed since legumes were grown, it may be necessary to inoculate. It is not definitely known how long legume bacteria will live in a soil. There is one case in which soy-bean bacteria have been known to live more than eighteen years in a fertile silt loam. Usually, however, the legume bacteria in a soil free from legumes decrease rapidly after two or three years. The only safe method is to make sure that the plants are inoculated. This can readily be done by digging up the roots and examining for the nodules. If plants

from various parts of the field show a considerable number of nodules it is safe to assume that the plants are well inoculated.

It is argued by some that it is well to inoculate all legumes unless it is definitely known that the specific soil is well inoculated, for it is maintained that "cultures," when applied directly on the seed, enable the young roots to begin drawing nitrogen from the air as soon as the plants can use it. This early nodule formation is important, as it gives the plant a start which may influence the rate of growth to the extent that it gives a greater uniformity of maturity. In cases where some natural inoculation occurs, or where uneven soil inoculation is applied, the plant roots must grow through the soil until they come in contact with bacteria. This causes a delay in growth which may be disastrous, besides resulting in a serious unevenness in maturity, especially with soy beans and peas."

Gains in Nitrogen.—It has been found that from fifty to 100 pounds of nitrogen is gained to each acre depending upon the specific legume grown, extent of inoculation, and the conditions of the soil. In some exceptional cases gains even up to 200 pounds per acre yearly have been reported. Where a soil is well supplied with nitrogen the legume even though inoculated feeds mainly on the soil nitrogen, whereas in soils poor in nitrogen the legume is forced to make use of its ability to obtain free nitrogen from the air.

If only 10 per cent of the 325,000,000 acres of soil yearly producing crops were cropped to properly inoculated legumes it would be a gain in nitrogen of over 8000 tons per year. The extent to which this would enrich the soil would depend upon the distribution made of the legume. If it were used as green manure the soil would be enriched to this extent. If fed and the manure carefully conserved and returned to the soil the soil gains in nitrogen would be from one-half to three-fourths this amount. However, where the legume is completely harvested and not even the manure returned, the gains in soil nitrogen would be small as the quantity in the roots and plant residues would not exceed that obtained from the soil. There are numerous cases on record where properly infected legumes have been used as green manures with average annual gains of soil nitrogen from fifty to 200 pounds. It is not as great where the legumes are fed and the manure returned to the soil, but, under this latter condition, the cost of the added nitrogen is considerably less.

CHAPTER XVII

THE PHOSPHORUS CYCLE

Nitrogen occurs primarily in the free form; phosphorus only in the combined. Nitrogen is very inert, phosphorus very active. The one always tends to pass into the elementary form, the other into the complex. Bacteria are essential in the liberation of each from various organic compounds. Organic nitrogen they change into nitrates, elementary nitrogen into proteins; organic phosphorus they transform into the inorganic, the insoluble into the soluble. The nitrogen cycle is complex and has been extensively studied; the phosphorus cycle is probably less complex and has received less careful attention.

Occurrence.—In comparison with some elements phosphorus is rare. It is twelfth in the order of abundance, as it constitutes only about 0.14 per cent of the earth's crust. Even this is very unevenly distributed. Some virgin soil contains enough for only a limited number of crops, whereas others contain sufficient for hundreds of crops. It also occurs in large natural deposits. It exists primarily as phosphates both in the soil and the natural deposits. The phosphorus resources of the United States are superior to those of any other nation. Although it is well distributed East and West, the bulk of it occurs in Utah, Idaho, Wyoming, and Montana. In these states are billions of tons of high-grade phosphate. The tendency is to mine and ship most of the highest grade to foreign countries. This is a wrong practice, because in the future it will be needed at home for the production of food for the ever-increasing population.

Next to nitrogen, phosphorus is the limiting element of crop production in most soils. Furthermore, the nitrogen supply is inexhaustible since it forms four-fifths of the earth's atmosphere and can be obtained cheaply by straight processes of industrial chemistry, or, better still, by the nitrogen-gathering bacteria. On the other hand, Hopkins has calculated from thousands of analyses that the average supply of phosphorus in the two million pounds of soil covering an acre of land to the usual plowed depth of six and two-thirds inches is only 2,200 pounds,

or enough for one hundred thirty crops of corn at the rate of one hundred bushels per acre.

Phosphorus in organic union is an essential constituent of all living protoplasm. It occurs in every cell, both plant and animal. Nucleoproteins (which constitute such a large part of the cell nucleus) on hydrolysis yield nuclein, and nuclein contains as high as 10 per cent of phosphorus. It constitutes over 20 per cent of the ash of corn, 19 per cent of the ash of wheat, 12 per cent of the ash of barley, and over 9 per cent of the ash of oats; hence, there are millions of pounds of it in each yearly harvest. The mineral part of animal bones consist largely of tricalcium phosphate. In 100 pounds of raw bone there is approximately ten pounds of phosphorus. An adult contains in his body about one and one-half pounds of phosphorus. If we allow one-half of this amount for each individual it would mean that there are approximately 700,000 tons of it tied up in the bodies of individuals living upon the earth at the present time, to say nothing of the far greater quantity bound up in the bodies of animals. Thus, it may be seen that phosphorus follows close to nitrogen after carbon, oxygen and hydrogen as a structural material in biological chemistry.

Outline of Phosphorus Cycle.—The mineral phosphates of the soil are rendered soluble through bacterial activity. This is taken up by the living plants and deposited either as organic or inorganic phosphorus compounds within the plant tissues. The plant tissues, if eaten by animals, yield phosphorus to the animal which is laid down in the body of the animal in the form of organic or inorganic compounds. The excreta of animals always contain phosphorus in both organic and inorganic forms. The inorganic phosphorus is readily utilized by plants and again starts on its cycle. However, the organic and animal residues must be mineralized by bacteria before they can be utilized again by plants. Microörganisms split off the carbonaceous material and the phosphorus is liberated mainly in the form of phosphates. Under some conditions mold action may give rise to small quantities of phosphine which must be oxidized again before becoming available to higher plants. In either event, the resulting phosphate is now ready to start once more on its cyclic journey through plant and animal organisms.

Mineralization and Solvent Action of Bacteria.—There are varying amounts of organic phosphorus in soil. These occur in the form of lecithin, phosphoproteins, and nucleoproteins.

Little has been done to determine the action of bacteria upon these compounds, but it is to be expected that they would be

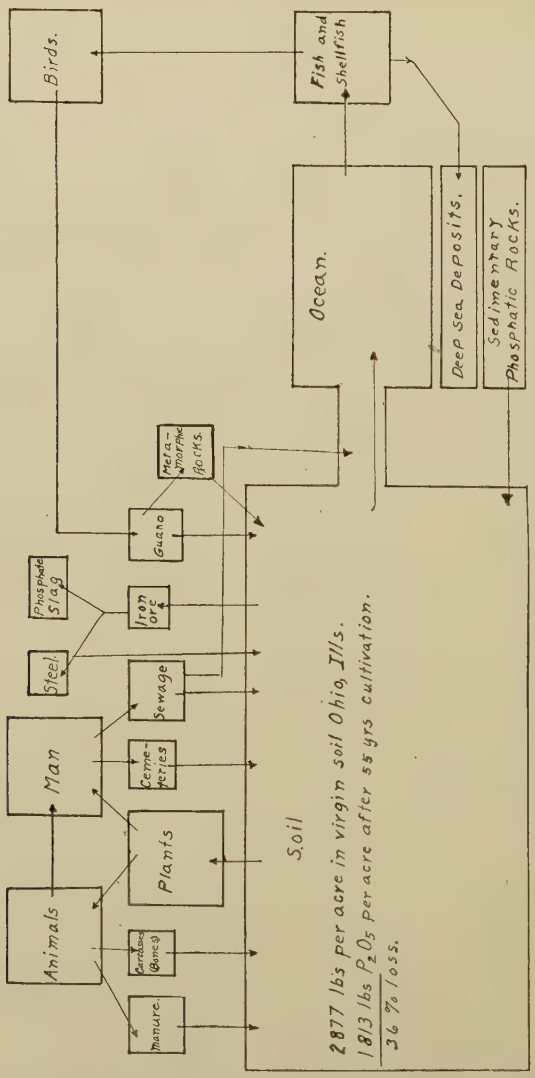


FIG. 56.—Illustrating the phosphorus cycle in nature. (*After Lotka.*)

hydrolyzed by bacteria as they are by ferments. Lecithin yields on hydrolysis glycerin, two molecules of fatty acid, phosphoric acid, and choline.

The phosphoproteins yields on hydrolysis amino-acids and phosphoric acid, whereas hydrolytic cleavage produces from nucleoproteins carbohydrates, phosphoric acid, purine and pyrimidine bases, with the intermediate formation of nucleins and nucleic acid, as may be represented by the following scheme:

nucleoproteins			
proteins		nucleins	
proteins			nucleic acid
carbohydrates pentoses hexoses unidentified	phosphoric acid	purine bases adenine guanine xanthine hypoxanthine	pyrimidine bases thymine cytosine uracil

Schittenhelm has shown that nearly all of the nuclein substances of feces disappear as they undergo autoputrefaction. He and Schroeter showed that bacteria may bring about a deep cleavage of yeast nucleic acid. Plenge showed that some bacteria have the power to liquefy the sodium salt of nucleic acid from thymine.

It seems reasonable, therefore, to believe that phosphorus is liberated by soil bacteria in a somewhat similar manner. It is known that the bacterial flora of the soil play a highly important rôle in rendering the phosphorus of the inorganic phosphates available to the higher plant.

Brown found that twelve out of twenty-three bacteria isolated from soil exerted a definite solvent action on plant-food that was almost insoluble, or only partially so. One organism which produced no gas but a large amount of acid showed the greatest solvent action upon calcium carbonate, whereas other organisms which produced gas—largely carbon dioxide—but not as much acid as the former, gave an action more marked than that of the stronger acid-producer upon the dicalcium and tricalcium phosphates. *B. subtilis*, *B. mycoides*, *Proteus vulgaris*, *Bact. coli commune*, as well as several agar cultures from garden soil, were found to be capable of dissolving the phosphates of bone and to a less extent the mineral phosphates. The greatest solvent action was exerted in media containing sodium chloride, potassium sulphate, and ferrous sulphate. As suggested by Kröber, the life activity of the bacteria, that is, assimilation of phosphorus by the living organism, probably plays little or no direct part in

dissolving the phosphates, but it is due to the action of the organic acids and of the carbon dioxide produced.

The acids produced by bacteria act upon all phosphates, converting them into the soluble monophosphate, but the rate of solution varies widely with the different phosphates. Tricalcium phosphate in precipitated form, dicalcium phosphate, and tetracalcium phosphate of Thomas slag are much more rapidly dissolved than the crystalline or the so-called amorphous phosphates. The solution occurs most rapidly in soils containing large quantities of organic matter due to the active fermentation taking place in such soils.

Grazia considers enzyme action to play a part in the dissolving of phosphates in soil. He had found that the addition of chloroform to a soil reduces bacterial activity and decreases the acid produced, at the same time increases the solution of phosphates. It is also known that the presence of ammonium chloride and sulphate in the cultural media is especially effective in increasing the solvent action of bacteria. The successive steps in the solution or decomposition of phosphorus compounds by bacteria are considered by some to be: (1) generation of acids; (2) secondary reactions in the solution; and (3) production of a soluble phosphorus containing organic substance. The first two of these are the result of the activity of the bacteria on the phosphorus, and the last is due to the metabolic assimilation of the microörganisms.

Hopkins and Whiting find that neither ammonifiers, nor true nitrifiers liberated appreciable quantities of soluble phosphorus. They considered the chief importance of the nitrite bacteria to lie in the fact that they render phosphorus and calcium soluble by the oxidation of ammonia into nitrites, the resulting nitrous acid reacting with the raw rock phosphate, and thereby rendering it soluble. The actual ratio found shows that about one pound of phosphorus and about two pounds of calcium are made soluble for each pound of nitrogen oxidized. In addition to the action of the acid radicals associated with the ammonia, the carbonic acid plays an important part. This may occur in soil poor in calcium carbonate, but Kelley has shown that in those rich in calcium carbonate there are only small quantities of phosphorus liberated. In the soil where the soluble phosphorus is being rapidly removed by the growing plant, or even by bacteria, it is certain that the various soil microörganisms play an important part in rendering phosphorus soluble. Results obtained at the Utah Experiment Station show that there is a relationship between

the increased nitrification produced by various salts, and the quantity of water soluble and organic phosphorus in the soil. This is illustrated by the following results which give the nitric nitrogen, water soluble and organic phosphorus in a soil after various treatments, the untreated soil being considered as 100 per cent.

PER CENT NITRIC NITROGEN WATER-SOLUBLE AND ORGANIC PHOSPHORUS OCCURRING IN SOIL RECEIVING VARIOUS SALTS

Treatment	Nitric nitrogen, per cent	Water-soluble phosphorus, per cent	Organic phosphorus, per cent
None	100.0	100.0	100.0
312×10^{-7} mol. $MgSO_4$	101.2	105.2	111.6
26×10^{-7} mol. $Fe_2(SO_4)_3$	102.0	94.3	142.3
625×10^{-7} mol. $Ca(NO_3)_2$	102.0	114.3	97.5
156×10^{-7} mol. KNO_3	106.4	108.1	103.3
625×10^{-7} mol. KCl	106.5	105.8	107.3
312×10^{-7} mol. $Mg(NO_3)_2$	106.5	115.5	95.1
125×10^{-6} mol. $MnCO_3$	108.4	107.5	162.6
156×10^{-7} mol. $MnCl_2$	112.9	100.2	98.4
78×10^{-7} mol. $MnSO_4$	113.2	94.3	107.9
13×10^{-4} mol. $FeCO_3$	117.4	105.6	94.8
25×10^{-5} mol. $MgCl_2$	123.2	109.7	96.5
625×10^{-7} mol. $Mn(NO_3)_2$	125.4	84.8	87.9
84×10^{-5} mol. $FeCl_3$	128.3	105.6	94.8
25×10^{-5} mol. $MgCO_3$	140.7	98.2	72.2
1×10^{-3} mol. NaCl	142.0	109.3	138.7
1×10^{-3} mol. $CaCl_2$	167.2	114.9	88.2
2×10^{-3} mol. $CaSO_4$	196.7	73.5	103.3

It is certain that soil microörganisms in their metabolism transform soluble inorganic soil constituents into either soluble or insoluble organic constituents. This is especially true of phosphorus which is found in the ash of bacteria in large quantities. The phosphorus, on the death of the organism, is probably returned to the soil in a readily available form, for Stoklasa has found that 50 per cent of the nitrogen of *Azotobacter* is nitrified within six weeks, and there is no reason for believing that the

phosphorus is liberated more slowly. Furthermore, there is the possibility that much of the mineral matter of the bacterial cell may become available, through the action of autolytic enzymes without the intervention of other bacteria.

It is further evident that an organism which possesses the power, when growing under appropriate conditions, of generating 1.3 times its own body weight in carbon dioxide during twenty-four hours, as does the *Azotobacter*, must greatly change the composition of the media in which it is growing. Water charged with carbon dioxide is a universal solvent and will attack even ordinary quartz rock. Granite and rocks related to it are also quite readily attacked, with the liberation of potassium and other elements. Carbonated water acting upon the tricalcium phosphate of the soil would form more readily soluble phosphates, for tricalcium phosphate is four times as soluble in water charged with carbon dioxide as it is in pure water.

Moreover, the nitrogen-fixing organisms form, among other products, formic, acetic, lactic, butyric, and other acids. The kind and quantity of each depends upon the specific organisms and upon the substance on which they are acting. These substances are sure to come in contact with some insoluble plant-food which they may render soluble. They have an especially highly solvent action on the insoluble phosphates. The resulting salts of calcium on being further attacked by bacteria would result in the formation of calcium carbonate.

Whether these processes give rise to an increase in the water-soluble plant-food of the soil depends upon whether the products of the second, the analytic reactions, exceed the products of the first, the synthetic reactions. It must not be forgotten that, although many of the organic phosphorus constituents may not be soluble in pure water, they may be more available to the living plant than are the constituents from which they were at first derived through bacterial activity.

This being the case, variations in the results reported from laboratory tests are to be expected. Stoklasa found that bacterial activity rendered the phosphorus of the soil more soluble, whereas Severin, in his early work, found the opposite to be true. Others have found that the solvent action of bacteria on insoluble phosphates is in direct proportion to the quantity of acid secreted by the organism.

In a later work, Severin obtained different results. He used three soils: one sterile; a second sterilized and inoculated

with cultures of *Rhizobium leguminosum* and *Azotobacter*. The solubility of the phosphorus in the last two was from 8 to 14 per cent higher than that in the sterile soil.

Although the metabolic activity of *Azotobacter* gives rise to large quantities of phosphate solvents, yet these organisms transform phosphorus into organic phosphorus compounds less rapidly than do the ammonifiers. There are, however, cases in which bacterial activity has decreased the water-soluble phosphorus of the soil and of raw rock phosphate. This does not mean, however, that it is less available; for, as pointed out by Truog, the mixing of floats with manure causes an immediate decrease in the solubility of the phosphorus in 0.2 per cent citric acid solution, yet when the thorough mixing is carried on within the feeding area of the soil the availability of the phosphorus is increased to such an extent that some species of plants are apparently able to secure almost an adequate supply of it from floats. The addition of manure to a soil greatly increases the carbon dioxide production, and for a short time measurably increases the solvent action of floats. Where there is for a time a decrease of water-soluble phosphorus in fermenting media, it is probably due to the formation of phosphoproteins within the bodies of the bacteria which later may be rendered soluble either by further bacterial activity or by autolytic enzymes.

The work of Lipman and his co-workers fully demonstrates that sulfur when added to the soil is oxidized into sulfuric acid which under appropriate conditions renders phosphorus available. The forty or fifty pounds of sulfur which reaches each acre of soil yearly, and also, the quantities which are being carried to it in plant residues must play some part in rendering plant-food available.

Losses of Phosphorus.—Phosphorus, when rendered soluble may, (1) be taken up by growing plants; (2) be reverted to insoluble phosphates and hence remain in the soil; or (3) be carried out by the drain waters into rivers, lakes or oceans. As the plants are eaten by the lower animals the major portion of the phosphorus is returned to the soil. This is not true of that consumed by mankind as most of it finds its way into the sewer. Van Hise estimates that the annual loss of phosphorus from this source is about 176,000 tons. The quantity annually locked up in the bodies of individuals who die in the United States is estimated by Lotka at 1105 tons. The quantity which is lost through leaching is even greater, as Blackwelder

writes: "Of the vast quantity of mineral matter annually delivered to the sea by the run-off, it is estimated that 1.45 per cent consists of phosphorus pentoxide. Using the best available figures for the amount of water thus brought to the ocean annually, it is calculated that if the phosphatic material in the form of solid tricalcium phosphate were loaded into standard railroad cars it would fill a train stretching continuously from Boston to Seattle and would be 7 to 12 times as great as the world's total production of phosphatic rock in 1911."

When the phosphorus reaches the ocean, nature begins its concentration. "In the sea water the blue-green algae concentrate phosphorus. Certain mollusks or crustacea feed on the algae, and other meat-eating mollusks devour the vegetarians. Small fishes eat the mollusks, large fish eat the small, finally seals and birds swallow the fishes, and so on in about six transformations the phosphorus originally contained in the sea water may come to rest in deposits of guano on desert islands or in accumulations of bones of vertebrate denizens of the sea." After concentration terrestial disturbances may elevate it above the water where it may be mined by man and used as a fertilizer on his soil.

CHAPTER XVIII

THE SULFUR CYCLE

The sulfur and nitrogen cycles have much in common. Both of these elements journey through earth, air, and water. Native nitrogen occurs in the atmosphere, native sulfur in soil and water. Both find their way into the atmospheres through combustion and decay. The supply in the soil comes from the atmosphere, but the quantity and nature of the products in the two cases are far different. Nitrogen occurs in the atmosphere in limitless quantities and can be used by the plant only after bacteria have caused it to combine with other elements. Sulfur is there in traces, only. The rains wash it into the soil where it is soon rendered available to the growing plant. When the supply in the soil of either of these elements becomes insufficient, they must be added in the form of fertilizers.

Occurrence of Sulfur.—Sulfur constitutes 0.08 per cent of igneous rock. It is estimated that 0.12 per cent of the earth is sulfur, where it occurs in both the free and combined form. Large deposits of free sulfur are found in Sicily, Japan, Louisiana, Texas, and other parts of the United States. Soil contains it in rather small quantities both in the organic and inorganic forms. Inorganically, it occurs mainly as sulfates combined with calcium, magnesium, sodium and other common bases. Its origin is the native rock, organic manures, and rain water. It is emitted in all volcanic regions, and sulfur springs contain it in large quantities.

Sulfur is one of the ten essential elements of plant-food. It is necessary for the formation of certain essential oils and enters into the composition of the bodies of all plants and animals. The quantity is small and varies with different plants, as may be seen by the following results of Hart and Peterson:

Crops	Size of crop per acre	Sulfur in plants	
		Per cent	Lbs. in crop
Alfalfa hay	8 tons	0.287	45.92
Red clover	4 tons	0.164	13.12
Corn grain	100 bushels	0.170	9.52
Corn stover	3 tons	0.126	7.56
Oats	100 bushels	0.189	6.05
Oat straw	2.5 tons	0.195	9.75
Timothy	3 tons	0.190	11.40
Wheat	50 bushels	0.170	5.10
Wheat straw	2.5 tons	0.119	5.95

It has been found at the Utah Experiment Station that the quantity in grains increases with the irrigation water applied during their growth. This increase is mainly inorganic sulfur; hence, it would have no food value to higher animals, inasmuch as they can use only organic sulfur.

Properties of Sulfur.—All have seen the familiar yellow sulfur either as brimstone or as the flowers of sulfur. It is without a marked taste and possesses only a faint odor. It is insoluble in water; when heated it melts, the nature and consistency of the product depending upon the temperature. Its great value resides in its ability to form compounds with other elements. It combines with oxygen yielding gaseous disinfectants. It forms strong acids which readily react with basic substances, thus, yielding available plant-food in the soil; when oxidized, it yields energy which may be used by certain microörganisms. Hence, we find bacteria continually at work changing it from the gaseous to the solid and from the solid to the gaseous form.

Outline of Cycle.—Sulfur is a constituent of the bodies of plants and animals, and as these putrify or decay it finds its way into the atmosphere. The combustion of coal yields large quantities. Enormous quantities of hydrogen sulfide and sulfur dioxide are emitted by volcanoes. These substances may be oxidized and washed from the atmosphere in the rain water. In the soil the products are worked over by bacteria and combine with the bases to yield sulfates. These may be taken up by the growing plant or leached out by waters and wander to the sea. "The quantity of sulfates discharged into the sea is enormous,

and we may well wonder why the sea is not mainly a sulfate solution. As it is, it contains in the dissolved salts 7.69 per cent of the sulfate radical. The reason is that it is being continually reduced to sulfides. These react with iron with the production of iron sulfide. Evaporation of the sea water or lake water in closed basins precipitate calcium sulfate at an early stage and there results our deposits of gypsum. From these, possibly through bacterial action, there results sulfur. A large part of the sulfur of the world is then continually in movement, changing from sulfide to sulfate with local reversion to native sulfur and from sulfate back to sulfide again."

The sulfur taken up by the growing plant is built into the plant structure, later decomposed by the animal, or becomes a part of

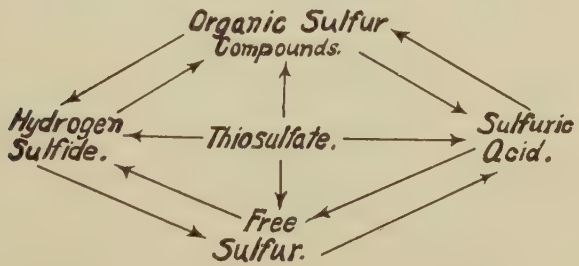


FIG. 57.—Illustrating the sulfur cycle in nature.

its tissue. The sulfur of the plant and animal tissue may be liberated through combustion or by bacterial action and again start on its journey.

Rôle of Sulfur Bacteria in Early Geological History.—Bacteria which possess the power of assimilating carbon dioxide in the absence of sunlight are known as autotrophic bacteria. The energy necessary for this assimilation is obtained from the oxidation of simple inorganic substances. The nitrite, nitrate, and certain sulfur bacteria are the only bacteria truly autotrophic. The methane, hydrogen, and especially the iron bacteria, while they possess the ability to obtain their energy from these simple compounds, may also use more complex organic substances as a source of energy. These are known as heterotrophic bacteria.

The sulfur bacteria, then, like the nitrifying bacteria, can live upon the bare rock. They obtain their carbon from the

atmosphere and function best in a concentration of carbon dioxide greater than is found in the atmosphere today. They must have found ideal conditions in the heavily-laden atmosphere of early geological periods. They obtain their nitrogen and energy from the ammonia and sulfur brought to them by the rain waters. They oxidize sulfur to sulfuric acid which reacts with the rock constituents and render them available. Investigations clearly indicate that the autotrophic bacteria can live on very simple inorganic compounds and build from them complex organic materials which later may be used by other organisms. It is quite likely that the sulfur bacteria, together with other autotrophic microörganisms, were the pioneers which gained a precarious foothold on the bleak, primitive rock and rendered it a fit abode for higher plants and animals.

Sulfur Bacteria.—The so-called sulfur bacteria have been divided into five groups which vary widely morphologically and physiologically:

(1) Colorless, thread-forming bacteria, which accumulate sulfur within their cells. They consist of three genera:

(1) Beggiatoa—motile, forming no sheaths,
(2) Thiothrix—fastened, forming no sheaths,
(3) Thioploca—thread-forming bacteria, surrounded with a jelly-like sheath.

These bacteria oxidize hydrogen sulfide and accumulate sulfur in the form of small spheres, consisting of soft amorphous sulfur which never crystalizes in the living cells. The sulfur is oxidized to sulfuric acid, which is immediately transformed into sulfates by the carbonates present. In the absence of sulfur, the organisms soon die. They require oxygen and ammonium salts but function in the absence of organic matter.

(2) Colorless, non-thread-forming bacteria, accumulating sulfur within their cells. There are several members of this group which have been studied. They differ from the members of the first group in that they do not form threads. They are found in the muds and sulfur water.

(3) Purple bacteria, oxidizing sulfur and accumulating it within their cells. They are distinguished from the other sulfur organisms by a red, reddish violet or reddish brown pigment which is unevenly distributed throughout the cell. In addition to the red pigment (*bacteriopurpin*), they also contain a green

pigment (*bacteriochlorin*). They are found abundantly in sulfur springs and in mud waters.

(4) The fourth group of sulfur bacteria includes colorless organisms that do not accumulate sulfur within their cells. The two members of this group which have been studied are *Thiobacillus thioporus*, an aërobic organism, and *Thiobacillus denitrificans* which is an anaërobe. They act on hydrogen sulfide and the sulfides with the production of free sulfur which never accumulates within the cell, although an abundant quantity accumulates outside and not in direct contact with the cell.

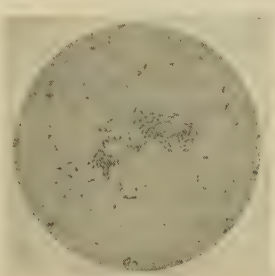


FIG. 58.—Thiobacillus thiooxidans. (*From "Principles of Soil Microbiology" by S. A. Waksman, Williams and Wilkins Co., Publishers.*)

(5) The fifth group oxidizes elementary sulfur and does not accumulate any sulfur either within or without the cell. The only member of this group which has been so far studied is *Thiobacillus thiooxidans.*

This organism was first obtained in pure culture by Lipman and co-workers by repeatedly transferring it to fresh media having a high acidity (PH 2.0–3.0). Later Waksman grew it on a solid medium of the following composition:

Sodium thiosulfate	5.0 grams
Monopotassium acid phosphate	3.0 grams
Ammonium chloride	0.1 grams
Magnesium chloride	0.1 grams
Calcium chloride	0.25 grams
Agar	20.00 grams
Distilled water	1,000.00 cc.

It is a short rod with rounded ends, usually occurring singly, occasionally in pairs, but rarely in triplets. The majority of these organisms are less than 1μ long and about 0.5μ in diameter.

Spore formation does not take place. The majority of the cells are non-motile. The organism stains well with gentian violet and methylene blue, and it is gram positive.

This microörganism is of especial interest for the following reasons:

(1) It, like other autotrophic bacteria, can utilize directly the carbon dioxide of the air. The energy comes from the oxidation of elementary sulfur.

(2) It functions in the complete absence of organic food.

(3) It is able, not only to tolerate, but to produce higher concentrations of acid than any other known living organism.

Sulfur Oxidation in Soil.—Whenever sulfur finds its way into a normal soil, it is slowly oxidized to sulfuric acid. But when this same soil has been inoculated with *Thiobacillus thiooxidans*, the sulfur is rapidly oxidized directly to sulfuric acid. The acid produced acts upon insoluble soil constituents such as calcium and magnesium carbonate, calcium silicates, and tricalcium phosphate and brings them into solution. Under appropriate conditions the reaction may render available appreciable quantities of plant-food.

The ratio between the sulfur oxidized and the carbon assimilated is about 32, but this ratio varies widely, depending on the conditions of growth. The variation is larger in the presence than in the absence of nitrates and glucose. When thiosulfate is used as the source of energy, the ratio is about 65. About 6.5 per cent of the energy made available is utilized by the organisms. This is a somewhat greater efficiency than is obtained with the nitrite and nitrate bacteria, for the sulfur bacteria are more efficient engines than most living cells.

The sulfur of the plant and animal residues which finds its way into the soil is broken down and liberated as sulfur dioxide, hydrogen sulfide, or elementary sulfur. These compounds in turn are transformed by the various sulfur organisms into sulfates and made available for the use of the higher plants.

Soil Losses of Sulfur.—The end products of the sulfur cycle in soils are sulfates, all of which are soluble. These may be removed from the soil by the growing plant or by drain water. The quantity removed by the plant depends upon the kind and size of crop grown on the soil. Alfalfa, cabbage, turnips, and onions remove comparatively large quantities of sulfur, whereas the grains, corn, oats and wheat remove smaller quantities. This may be seen by the following table of Hart and Peterson:

SULFUR REMOVED BY DIFFERENT CROPS

Crop	Dry weight, pounds	Sulfur removed, pounds
Alfalfa	9,000	26.0
Red clover	3,763	6.2
Sugar beets		
Roots	4,320	3.8
Tops	1,848	8.0
Total crop	5,168	11.8
Turnips		
Roots	3,126	23.1
Tops	1,531	13.8
Total crop	4,557	36.9
Cabbage	4,800	39.2
Corn		
Grain	1,500	2.6
Stalks	1,877	2.2
Total crop	4,377	4.8
Oat		
Grain	1,625	3.0
Straw	2,353	4.9
Total crop	3,978	7.9
Wheat		
Grain	1,530	2.7
Straw	2,653	3.7
Total crop	4,083	6.4

At this rate 400 pounds of sulfur in the soil would be sufficient for about 15 crops of alfalfa, 10 crops of cabbage, or 63 crops of wheat. It has been found that the soil of the Greenville Experimental Farm (Logan, Utah) contains only 247 pounds of total sulfur in the surface acre-foot. This would be sufficient to meet the needs of 39—50-bushel crops of wheat, 31—100-bushel crops of corn, or 38—75-bushel crops of barley grain at the rate these grains are at the present time actually taking the sulfur from the soil. It is doubtful whether the sulfur content of the average soil of the United States contains over 400 pounds per acre of sulfur. Hence, it is evident that the sulfur actually stored in the

soil at a given time is sufficient to produce crops for only about one generation of human beings.

The second and greater loss of sulfur comes from the leaching-out of the soluble-sulfur compounds by drain waters. Many factors influence this loss; namely, the kind of soil, the amount of precipitation, system of cropping, and methods of cultivation. The greatest loss comes during the season of the year when no crop is growing on the soil and when the soil is augmented by everything which tends to loosen and aërate it. The actual loss of sulfur in some soils has been found to be as low as 8 pounds per acre yearly, but in others as high as 281 pounds. Some investigators have noted a loss of from 40 to 50 per cent of the total sulfur of the surface soil within a period of fifty years. The reduction would be even greater than this were it not for the fact that there is a compensating factor in the sulfur cycle.

Soil Gains in Sulfur.—Combustion and decay are constantly liberating gaseous sulfur compounds. Millions of tons of sulfur are thrown yearly into the atmosphere by volcanoes. This sulfur finds its way back into the oceans, lakes, streams and soils of the country. The quantity brought to the earth varies yearly in different localities. It is high near volcanoes and manufacturing centers where fuels rich in sulfur are used. Reports have been made of regions where the annual sulfur brought to the soil by rain water does not exceed 4 pounds per acre. However, in other localities as much as 131 pounds per acre is annually brought to the soil. The average yearly quantity brought to each acre of tillable soil is approximately 25 pounds. Were it not for this gain from rain waters the sulfur question would be more acute than the nitrogen problem.

Soils also gain sulfur from plant and animal residues. Each ton of farmyard manure carries to the soil about 3 pounds of sulfur and the quantity added in commercial fertilizers may at times be sufficient to actually increase soil sulfur in addition to replacing that removed by the growing plant and lost in the leach water.

Economic Consideration of Sulfur Cycle.—It is evident from what has been said that the sulfur organisms are essential for the life of higher plants and animals. Were it not for them, sulfur would be locked up in forms unavailable to higher life and eventually all of the available sulfur would disappear. However, before this could happen all forms of life would disappear, as sulfur is essential to the normal functioning of every cell.

Sulfur is oxidized to sulfuric acid by bacteria. The acid in turn reacts with the various insoluble constituents of the soil and renders them soluble. By this means the phosphorus and potassium are made available to the growing plant. The Lipman process for preparing soluble phosphorus is based on this principle. Raw rock phosphate and sulfur are mixed with some inert substance, inoculated with *Thiobacillus thiooxidans* and kept at optimum moisture and temperature conditions. In time there results acid phosphate which in the future may replace the present commercial phosphate. In this manner bacteria and sulfur replace the sulfuric acid used in the commercial process.

Preliminary tests indicate that the sulfur bacteria may be made to play an important rôle in the reclaiming of black alkali soil. Sulfur added to the soil is oxidized to sulfuric acid. This in turn reacts with the highly toxic sodium carbonate and transforms it to the less toxic sodium sulfate which, if necessary, may be leached from the soil.

Preliminary experiments indicate that in some soils it is possible, by the addition of sulfur, to change the reaction of the soil to such a degree that the development of certain pathogens is prevented without injuring the particular plant. In some cases of field tests it has been found possible by this method to decrease potato scab from 30 to 60 per cent.

CHAPTER XIX

BACTERIA IN RELATION TO OTHER ELEMENTS

Plants require ten elements for their normal growth and fruition. Animals require even more. These elements are built into complex compounds and hence become unavailable to other higher plants. We have learned how carbon, nitrogen, phosphorus and sulfur are transformed by bacteria, so that they again become useful to the higher plant. Let us now examine briefly the wanderings of a few of the other "life elements."

Iron.—Iron constitutes 4.5 per cent of the earth's surface. Aside from oxygen it is the most abundant of the "life elements" and is required by plants and animals in smaller quantities than is the case with any of the other elements. A 50-bushel crop of wheat contains 100 times as much nitrogen: 10 times as much phosphorus and 8 times as much sulfur as iron. Yet small as the quantity of iron may be, it is absolutely essential to the life and well-being of plants and animals.

Although iron does not enter into the composition of chlorophyll, it plays an important part, directly or indirectly, in the production of chlorophyll: for, if iron is withheld from the plant, the leaves do not become green. Later, if the iron is supplied, the chlorophyll soon appears. In the animals, iron plays a vital part. In the body of the human adult there are about 4.5 grams, contained for the most part, in the hemoglobin of the red corpuscles. The importance of iron arises from the fact that it plays the essential rôle of an oxygen carrier. It acts as a catalyst causing the transfer of oxygen from the air in the lungs to the various tissues of the body through the blood stream. Probably in many of the oxidation changes going on in the plant and animal cell, iron acts as the catalyst which speeds up the reaction.

Although iron is abundant in the soil and is required by plants in such small quantities, nevertheless, it is not uncommon to see plants in a chlorotic condition which indicates iron starvation. This may result from conditions being inimical to bacterial activity, consequently, the iron is not available.

Iron Bacteria.—There are living in the waters of certain muds and soils, bacteria which possess the power of abstracting iron from the water and depositing it on the surface of their bodies. It is to such organisms that the term "iron bacteria" is applied. They are of especial interest to the student of biology for two reasons: (1) Most of the organisms in this class are slightly higher in the scale of evolutionary development than are the majority of bacteria. They have taken the first step forward in what may be termed communal life. In a very primitive way there appears to be a division of labor within the cells. (2) They are intensely interesting because of their peculiar habit of depositing iron on their bodies. How and why this peculiar condition is brought about or possessed by these organisms is not fully understood. Then too, they are of special interest to the engineer, chemist, and geologist on account of their ability to multiply rapidly and deposit great masses of iron. The result of this action is seen in the great deposits of bog iron which have come down to us from earlier geological periods. Today they often make their presence known by the deposits of iron which they accumulate in water mains. This at times becomes a real nuisance.

Harder divides the iron bacteria into three principal groups: (1) Those which precipitate ferric hydroxide from solutions of ferrous bicarbonate, using the carbon dioxide set free and the available energy of the reaction for their life processes. (2) Those which do not require ferrous bicarbonate for their vital processes but cause the deposition of ferric hydroxide when either inorganic or organic iron salts are present. (3) Those which attack iron salts of organic acids, using the organic acid radicle as food and leaving ferric hydroxide, or basic salts that gradually change to ferric hydroxide.

These microörganisms belong to the group known as higher bacteria and differ from true bacteria in morphology and methods of reproduction. They occur as long threads which are usually composed of many individual cells and present a variety of forms such as single threads composed of cylindrical cells placed end to end and generally enclosed in a sheath, ribbon forms produced by the bending of the filaments in the middle and the twisting of the ends around each other like rope. They generally reproduce by the formation of conidia or swarm spores. They belong to the family Chlamydobacteriales. There are five genera: *Leptothrix*, *Didymohelix*, *Crenothrix*, *Sphaerotilus*, and *Clonothrix*.

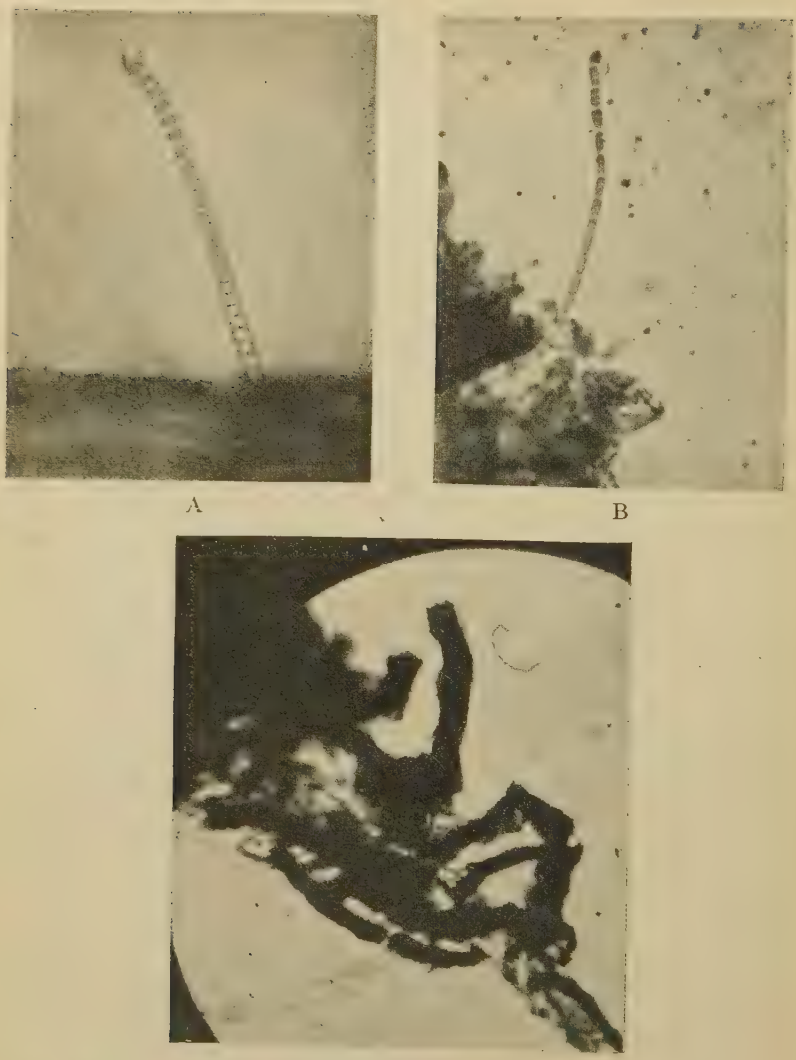


FIG. 59.—Iron bacteria (Crenothrix polyspora). A. From ferruginous water flowing into River Clyde. Condition slightly older than that depicted in B. Sheath is visible and open at the top. Cells in a single row. B. Semi-mature thread. Thickness $\frac{1}{20}$ millimeter. Cells visible without treatment. Apex closed. Cells in a single row. C. Basal parts of Crenothrix polyspora in state of decomposition. Described under the various names of Leptothrix meyeri and Megalothrix discophora. (*After Ellis.*)

Opinions differ as to how they withdraw iron from solutions. Some workers have maintained that the deposition of iron hydroxide is a vital process of the organism and as such is carried out directly by the living cell. Others hold that the process is chemical and is incidental and not vital to bacterial life. Still others hold that the accumulation is mechanical and that particles of iron hydroxide in the water are caught and held mechanically by the mucilaginous parts of the bacteria. Probably different genera act in different ways.

Iron Bacteria as Pests.—Some of the iron bacteria are so common and are such a nuisance that in many places in the United States and Europe they are known as the "water-pest bacteria." They will attach themselves to stonework, woodwork, and pipes in reservoirs and water-mains, and grow out into the water as fine threads. These continue to multiply until there results huge streaming masses of slimy material in which iron hydroxide is deposited. When the bacterial body disappears, there results a deposit of iron which, if on the inside of the water main, may result in clogging. They are, therefore, objectionable for a number of reasons: (1) They increase the amount of organic matter in the water, which in turn causes a great increase in the number of saprophytic bacteria in the water. (2) They decrease the bore of the conduit pipes, and cause a decrease in the velocity of the flow of the water and in time may result in a complete clogging of the mains. It is not necessary that the water be heavily charged with iron to bring about this condition for they grow well and absorb iron from water having only one part of iron in a million parts of water.

Two methods are in use for combating the action of the iron bacteria in reservoirs and water mains: (1) The organisms require ammonium salts for their growth and thrive in the presence of organic matter; hence, the removal of their food from the water will cause them to disappear. This is done by oxidization. Green plants may be grown. These liberate oxygen, and the nitrifying bacteria oxidize the ammonia to nitrates. (2) The iron bacteria grow best in an acid media; consequently, making the waters alkaline often prevents the growth of the pests and also accelerates the growth of nitrifying bacteria.

Bacteria in Relation to Iron Deposits.—Both chemical and biological processes have played an important part in the deposition of iron. In some deposits, chemical processes have predominated; while in others, bacteria have played a major

part. Iron bacteria must have flourished in the rich, peaty water of the past and taken from them the iron and deposited it within their sheath. Later decomposition destroyed their bodies and there resulted the iron deposits, many of which are being mined today.

Bacteria and the Transformation of Iron in Soil.—Iron has long been known to be an essential plant-food. Although it is not a constituent of chlorophyll, yet in the absence of iron, plants soon take on a yellowish or blanched appearance known as chlorosis. Due to the general distribution of iron in soils it has generally been believed that there is sufficient in soils for normal plant production, yet chlorosis often occurs in the plant. This raises the question: If iron be present in soil in sufficient quantities, is it there in the proper form for normal plant growth? It occurs in soils in both the organic and the inorganic forms. All of the organic and much of the inorganic iron is unavailable to the growing plant. The organic iron must be liberated by decay bacteria. During the process acids are produced which in turn may render the inorganic iron soluble. Ferrous iron is quickly oxidized by chemical and biological processes of the soil. Chlorosis may, thus, be an indication of slow or improper bacterial activities in the soil.

Stimulation of Bacteria by Salts.—Small quantities of most poisons when administered to man act as a stimulant, and it is only when the dose becomes large that the poisonous action is perceptible. This appears to be a universal law, holding for man, animals, and plants including bacteria, for it is found that when small quantities of alkali are added to a soil the bacterial numbers and activity increase. However, the extent of this increase varies with different microörganisms and also with the salt. Small quantities of the carbonates, chlorides, sulfates and nitrates of sodium, calcium, and magnesium greatly increase the beneficial bacterial activities of many soils. The increased bacterial activity may liberate more phosphorus and potassium for the plant. Calcium sulfate or gypsum is especially active in increasing nitrification, and increases proportionately the plants growing on that soil. The dealer in gypsum often takes advantage of this fact in advertising his materials by writing the word PLASTER with a heavy application of the material in large letters on a cultivated field in view of the public road. In the larger greener growth of grain crops or grasses the word PLASTER can be read by the passerby. In

this way the farmers are induced to use gypsum or land plaster. This, however, is usually a stimulant, and if the landowner continues to apply the material year after year where the word was first written, the time will come when it cannot be read. If he continues the application long enough, ultimately one may again read the word PLASTER, but this time the grain composing the word would be inferior to that surrounding it, and the physical properties of the soil, where the plaster has been applied will be found to be bad.

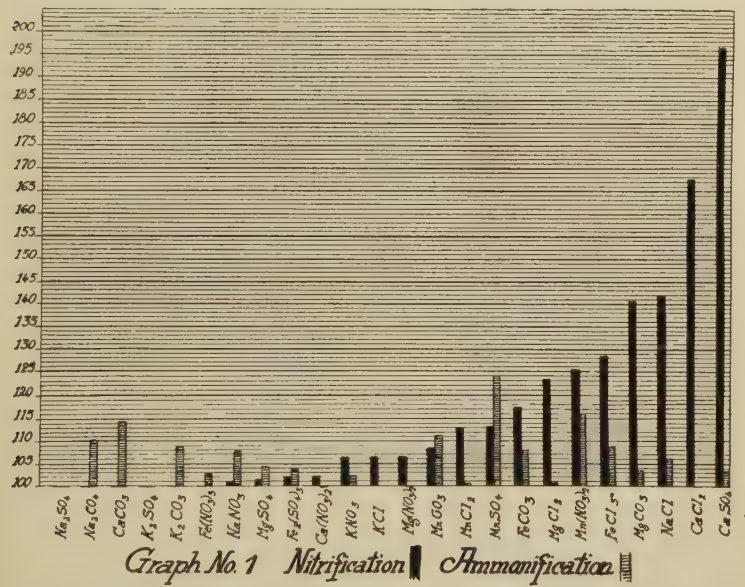


FIG. 60.—Illustrating the stimulation of bacteria by salts. The untreated soil is considered as 100 per cent.

Common salt is also often used as a crop stimulant. This mainly increases bacterial activity and with the exception of the gain in nitrogen it is also a stimulant. Now, it may be legitimate at times to use such substances, but the farmer should do so with the understanding that he is using a stimulant—not a plant-food—and that he is getting a temporary—not a permanent—effect.

Toxicity of Alkali Salts.—If we gradually increase the quantity of alkali salts in a soil, there comes a time when the bacterial

activities are decreased. The quantity of alkali which must be added to a soil to make it poisonous to bacteria varies with the salt, the soil, and the bacteria. The quantity, stated as pounds

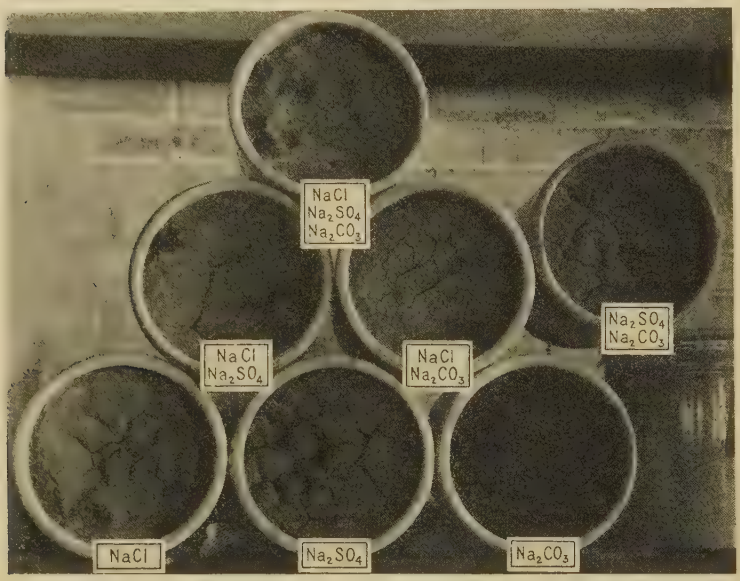


FIG. 61.—Appearance of alkali soil. (*From Greaves', "Bacteria in Relation to Soil Fertility." Published by D. Van Nostrand Co., New York.*)

per acre, which it was found necessary to apply to a calcareous loam in order to become toxic to ammonifying and nitrifying organisms, is given below:

Salt	Ammonifier (pounds per acre)	Nitrifier (pounds per acre)
Calcium sulfate	24,500	
Sodium carbonate	22,890	57
Sodium sulfate	2,556	79
Magnesium sulfate	2,200	270
Magnesium chloride	1,700	29
Sodium nitrate	1,530	47
Sodium chloride	263	
Calcium chloride	61	313

These results are not to be taken as indicating that neither ammonia nor nitrate is produced in a soil containing the above

quantities of alkali, but they simply indicate the concentrations at which the activity of the microörganisms are slowed down in this particular soil.

Ammonia is produced in a soil containing considerable alkali, but the production of nitrates is interfered with even when small quantities of alkali are present. The great difference between the reaction of the ammonifiers and nitrifiers is due in part to the fact that many molds ammonify, whereas it is only bacteria which nitrify. Molds are considerably more resistant to alkali than bacteria.

Much larger quantities of alkali than those reported above have to be in a soil before the nitrogen-fixing bacteria are injured. In an alkali soil the nitrifying bacteria disappear first, followed by the ammonifying bacteria; and finally, in the barren waste the nitrogen-fixing microörganisms disappear.

The first injury done to bacteria by alkali is due to an increased osmotic pressure of the soil solution, for the osmotic pressure of all these solutions at the point at which they become injurious is between 1.65 and 1.95 atmospheres. As larger quantities of the salts accumulate they poison the soil microörganisms. Thus, alkali salts are highly fatal to the nitrifiers, less so to the ammonifiers, and still less to the nitrogen-fixing microörganisms.

It is interesting to note that alkali salts are poisonous to nitrifying microörganisms in much lower concentration than to higher plants. It is quite possible that the early injurious effect observed when land starts to "go bad" from an accumulation of alkali is caused by the action of the salts on the nitrifiers. It is, however, only after these salts become quite concentrated that they actually injure the plant. Moreover, after a soil has been leached of its alkali it is a number of years before it becomes productive. This unproductive period is in a measure due to the bad physical condition of the soil, but this is not the only factor, for the addition of manure or extracts of productive soil greatly shortens the unproductive period by bringing to the soil the necessary microflora.

Variation in Toxicity.—The toxic or poisonous action of a salt, as already stated, varies not only with the kind and quantity of salt present but also with the soil. A quantity of common salt or other alkali which would be sufficient to greatly retard bacterial growth in a coarse soil may be without effect in one of fine texture. Moreover, a salt is more poisonous in a soil low in organic content than in one of high. It appears that it is the

greater surface exposed to the action of the salt in the fine-textured soil that holds the salt in such a way that the bacteria are not injured. This is spoken of as adsorption. Moreover, salts are less toxic where there is a mixture than where a single salt is present.

Antagonism.—If certain salt-water fish are placed in a solution of common salt having the same osmotic pressure as sea-water, they soon die. Death in this case is not due to a lack of food, as similar fish placed in distilled water live for some time. If, however a small quantity of calcium chloride is added to the first type of solution, the fish will live normally. Moreover, when the heart is removed from the body of a cold-blooded animal and placed in a salt solution it soon dies, but if a small quantity of calcium salt is present, the heart beats normally for some time. When one salt thus counteracts the poisonous effect of another it is spoken of as antagonism and a solution made up of a mixture of salts producing no toxic effects is known as a balanced solution.

The property of antagonism also manifests itself in plants. One may take a soil which contains sufficient common salt to be injurious to plant life and by adding calcium salts to it overcome the toxicity. This appears to be a general law, for the injurious action of sodium carbonate, sodium sulfate, and a number of other salts toward bacteria can be neutralized by the use of calcium salts. The extent to which this neutralization can be carried is limited, as it is only the less concentrated salts which can be thus neutralized. However, this does indicate that a concentration of a single salt is often more injurious than is a similar concentration of several salts. We, therefore, have the peculiar phenomenon of being able to reclaim some alkali soils by adding more alkali to them.

Numerous workers have attempted to explain this peculiar phenomenon. Even before ideas on antagonism had taken a definite form, Löew elaborated a theory to account for the toxic properties of magnesium when calcium was not present in sufficient quantities. He supposed calcium to be a necessary constituent of the chlorophyll bodies and nucleus, and that when magnesium is present in great excess that this takes the place in those bodies which should be occupied by calcium. This causes a structural disturbance on account of which the protein substances cease to be active and death ensues. It will be observed that this will only explain the need for a balance between calcium and magnesium and not between other compounds, as is often the case.

Osterhout, who has done much work in this field, considers that the value of calcium lies in its effect at the surface between the absorbing membrane and the external solution and is not due to chemical action inside the cell. He, therefore, explains antagonism by assuming that antagonistic substances prevent each other from entering the cell, for, according to Loeb, if the wrong salts reach the inside of the cell they replace the salts in the protein and the cell dies. Out of this work has grown the idea of physiologically balanced solutions, or as Osterhout puts it, "normal life is possible only when necessary salts combine with the colloids of living substance in a definite ratio."

CHAPTER XX

WATER

Next to life, what is the most marvelous thing in the world? Is it the diamond, the hardest and most brilliant of all gems which is so prized by the young? Is it gold for which some will give even life itself? Is it radium, which is so rare that a ton of pitch blend must be worked over to obtain only one gram; which incessantly, and without appreciable loss, pours off rays that travel at the rate of 20,000 miles a second, forty thousand times faster than a rifle bullet; which penetrates thick blocks of metal, and which has such mysterious and wonderful action on living matter that it is being utilized in the curing of a number of ailments? No, as we shall see, it is common water.

Importance of Water.—Water composes two-thirds of the human body. It enters into the makeup of every tissue. The muscles, which react so nicely to the will and perform such marvelous feats, contain 75 per cent water; the liver, which stands guard over the body, constantly protecting it from poisons, consists of 75 per cent water; the bones, which possess a tensile strength of 25,000 pounds per square inch and are one and one-fourth times as strong as cast iron, consist of 40 per cent water; the brain, the most complicated and wonderful organ of the body, consists of 85 to 90 per cent water; the blood, that cosmopolitan fluid which visits every tissue of the body bearing to it nutrients and from it waste, contains over 90 per cent water. All the secretions of the various digestive glands consist mainly of water, which is present not merely as a vehicle in which the active principles are conveyed, but it actually enters into practically every change through which carbohydrates, fats, and proteins pass in the process of digestion and metabolism. Each one hundred grams of fat burned in the body yields 107 grams of water. It is the fluid in which the mineral nutrients which play such a vital part in life phenomena are held. Water gives to the tissues plumpness, carries off waste, regulates body temperature, acts as a lubricant, and is a universal catalyzer.

The ancient philosophers recognized the importance of water, and Thales founded his philosophy and science on the idea that water is the origin of all things. The elements of the ancients were earth, air, fire, and water. Mathews stresses its importance to man in the following words:

"It is marvelous to find that living matter with all its wonderful properties of growth, movement, memory, intelligence, devotion, suffering, and happiness should be composed to the extent of 70 to 90 per cent of nothing more complex nor mysterious than water. Such a fact as this is most perplexing, especially when all experiments show that the water is playing a profoundly important part in the vital phenomena. Any interference with the amount normally present makes a change at once in the activities of the cell. In fact, we might say that all living cells function in water. Not only is this obviously true in the lower and simpler forms of animals and plants, which are little more than masses of naked protoplasm living in the water, but it is also true of the higher forms in which the internal medium, or environment is of a liquid nature; the lymph, the blood, or the sap is the immediate environment of the cells. Water is the largest and one of the most important constituents of living matter, and if organisms are carefully examined the most various devices are found to assure the regulation of the water content of the cells of the body."

Animals whose habitat is far removed from the sources of water possess special mechanisms for their protection during water deprivation. The camel has not only a system of stomachs effectively arranged for carrying its water-supply, but it has humps with great stores of fat which, when metabolized, yield large quantities of water. It also possesses a thick coat of hair which reduces evaporation to a minimum and a digestive tract which permits no waste of water. Man suffers little when dying from hunger as compared with thirst. At first, when deprived of water, the mouth and throat become parched, the saliva dry and sticky, the tongue clings to the teeth or the roof of the mouth, and there is a lump in the throat which causes endless swallowing. Later the eyelids stiffen, and the eyeballs assume a set sightless stare; delirium develops with visual illusions of lakes and running streams which are just beyond the reach of the individual, and in a short time the awful suffering is relieved by death. In starvation, an animal may lose practically all of its sugar and fat, half of its body proteins, approximately 40

per cent of its body weight, and still live. On the other hand, the loss of 10 per cent of the water content of the body results in serious disorders, and the loss of from 20 to 22 per cent results in death.

Nor do we have to confine our attention to biology when considering the importance of water. The physicist has chosen it to define his standards of density, of heat capacity, and as a means of obtaining fixed points on the thermometer. The chemist is often exclusively concerned with reactions which take place in water and which are often due to it. The geologist reads the history of the earth from the effect which has been produced by water. While controlled in the liquid, solid, or gaseous form, in the stream which turns the wheel of the electric generator, the ice which protects our food, or the steam which drives our engines, it is man's greatest friend: but, when uncontrolled in the raging torrent, or when congealing within or under a building, it becomes a relentless foe. Thus, considered from a physical, chemical, or biological viewpoint, water is the most important compound known to man.

Classification of Water.—Water may be classified as good, polluted, or infected. A good water, from a sanitary viewpoint, is one which will pass a satisfactory physical, chemical, and bacteriological analysis. This should also include a sanitary survey of the water-shed.

A polluted water is one containing organic wastes of either animal or vegetable origin. It may or may not be a dangerous water, but it is a suspicious water.

An infected water is one which actually carries germs which cause human diseases. Such water carrying the germs of disease is dangerous, but it is seldom that bacteriological analysis can prove their presence. . A drinkable water is sometimes referred to as a potable water.

Waters are very conveniently classified as to their immediate source, rain or snow, surface, and ground waters. The same water at different times plays different rôles; at one time it is in the atmosphere; later, on the surface of the earth; and still later, in the ground. In each case it is water, but from a sanitary viewpoint it has greatly changed. In the atmosphere, or just as it condenses, it may be nearly pure, but on reaching the earth it may become polluted, or even infected.

Atmospheric water may fall as rain, snow, hail or dew, and as such represents the purest natural water at our command. It

is water which has been distilled by nature on a gigantic scale. As it falls through the atmosphere it becomes charged with dust, soot, bacteria, nitrous, nitric, and sulfurous acids, the quantity and kind of pollution collected varying with the locality and the time which has elapsed since the atmosphere was last washed. On reaching the surface of the earth it is a safe water, although not free from foreign substances. Here, however, it quickly takes up more dirt, more organic matter, and more bacteria, some of which may be pathogens.

It is a well-known fact that some of the filthiest, if not the most dangerous water used for domestic purposes, comes from rain-water tanks. This is due both to the methods of collection and storage which pollute, but usually does not infect it.

Rain water is especially free from calcium and magnesium salts, hence is referred to as a soft water. It is especially valuable for cooking and laundering purposes. When the calcium and magnesium exist as a bicarbonate, heating breaks it down with the production of an insoluble carbonate. For this reason it is known as temporary hard water. Waters containing the chlorides and sulfates of calcium and magnesium cannot be softened by boiling and are known as permanently hard waters.

Surface waters include rivers, creeks, small streams, lakes, ponds, and reservoirs. Surface streams vary widely in purity depending upon the nature of the catchment basin and the season of the year. Streams which drain mountains or sparsely settled districts may be safe. Those which drain lowlands, especially those inhabited, are generally polluted and may be infected. The too general practice of running sewage into a nearby stream makes the latter a constant menace to health. The impossibility of protecting such waters from pollution is forcing the sanitarian to the conclusion that in as far as possible such waters should be purified before they are used.

Lakes, large ponds, and reservoirs, when fresh and kept free from pollution with human and industrial waste make admirable sources of water. They may be more easily protected than are streams and the natural agencies for purification—time, sedimentation, sunlight and enormous dilution—play a great part in freeing the water from any accidental pollution which may find its way into the water.

Ground waters are of two classes:

(*a*) Deep springs and artesian wells, from which most bacteria and suspensoids have been removed by filtration. Such waters

in passing through the soil may take up large quantities of carbon dioxide. Such water has a great solvent action for lime and other inorganic constituents; hence, they are usually safe but hard.

(*b*) Shallow springs and wells correspond more nearly to surface waters and are often polluted and at times infected. Shallow wells and surface springs, especially if in porous soil, may be polluted from toilets, or cesspools. The water from these seeps through the soil and carries with it the death-dealing microbe. Where the wastes are some distance from the well, especially where the soil is tight, the danger from this source is small as water may be purified by passing through a few feet of good, fine soil. In many cases, the little culprit journeys along the surface and into the water—not by its own volition, however, for these organisms are dependent upon man and the lower animals for their locomotion.

FIG. 62.—Improperly covered well, pollution can readily get in from the top.

Who has not seen the well, loosely covered with planks between which grass-hoppers, toads, and manure may find their way? It is easy to understand how filth from the boots of working men, or from children playing on the cover, or from poultry walking about carrying infection on their feet may get into the water of such a well. One need not go far in many rural districts to find the outhouse freely open at the back so that fowls or pigs can walk under and from there onto the planks covering the well. The filth left on the planks is soon washed into the well. This would do no harm, if the disease germs were not present. There may be no known cases of typhoid or dysentery on the premises, but in every community the carrier, like the proverbial poor man, is always with us, and sooner or later his infection will

be deposited in the outhouse and find its way to the well. The inhabitants learn of the presence of the disease germs when there is an out-break of disease. The old lumber which covers so many wells should be replaced by tight-fitting cement platforms.

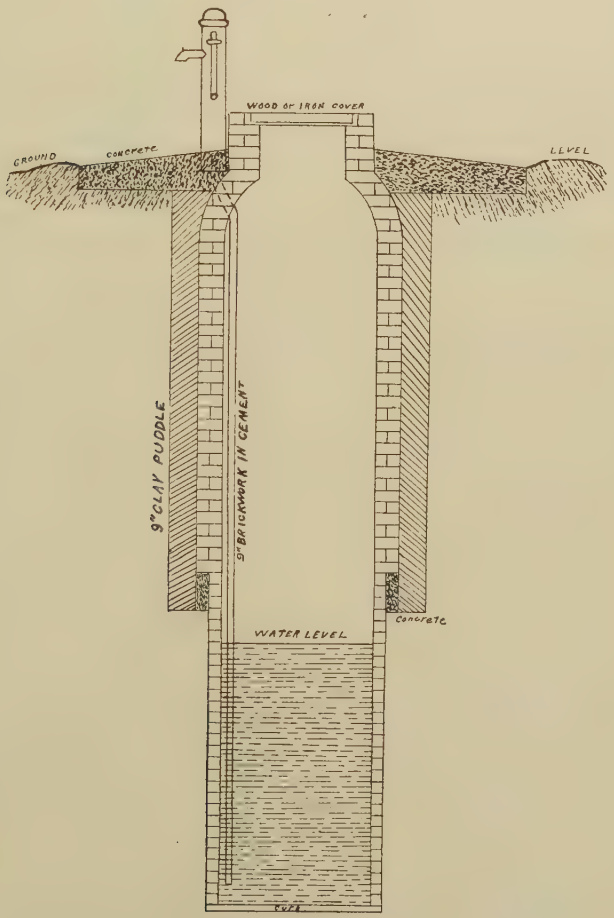


FIG. 63.—Properly constructed and covered well.

These should cover not only the surface but several feet of the soil surrounding the well and pass some three or four feet into the ground so that all the water which may find its way to the surface will have to pass through several feet of firm soil before it

reaches the drinking water. It is probable that if this procedure were generally practiced, 90 per cent of the wells which are now a constant menace to health would yield safe water.

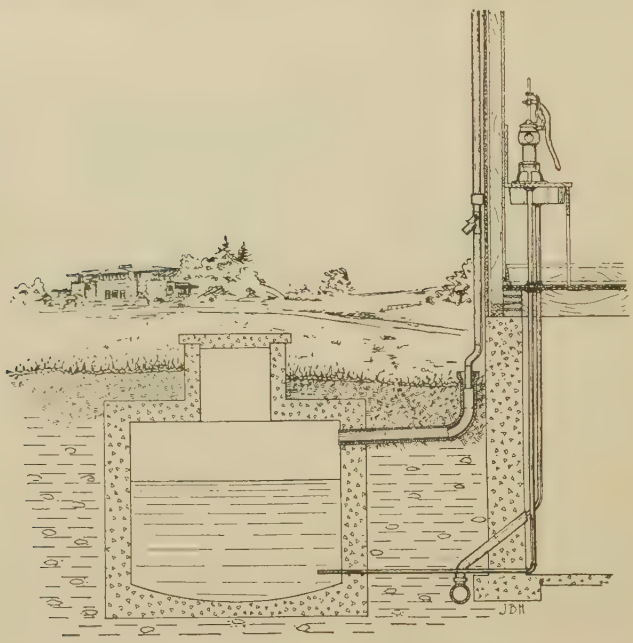


FIG. 64.—Properly constructed cistern located outside the house. (*Minn. State Board of Health.*)

How to Tell Good Water.—The deep wells and springs are usually safe. But how are we going to know when we have the deep spring or well? And how can we tell when pollution is finding its way into the water? Valuable information can be obtained on these questions by anyone who will apply a few simple tests.

In northern districts where there are great changes in temperature one often finds a spring, the users of which say, "The water of this spring is warm in winter and cold in summer." However, if the temperature of the water were taken with a thermometer during summer and winter, it is probable that it would be found to be the same on both occasions. A uniform temperature indicates that the water is not coming directly from the surface and, therefore, is not likely to be polluted.

Even clear, sparkling water may be laden with death-dealing germs; hence, brilliancy and clearness do not insure purity. Yet that well or spring water which becomes turbid after a storm, if in the neighborhood of human habitation, is dangerous. The storm which carried into the water clay mud and silt may also carry human filth, and this perchance may be the resting place of disease germs.

All ground waters when evaporated leave a residue. The nature of the residue varies with the water. Water free from pollution may give a voluminous white deposit when evaporated. When this deposit is dark it indicates organic matter. The odor of this dark mass when charred in the vessel in which evaporation occurs, may indicate the nature of pollution. That which comes from corrals and outhouses has an offensive, fetid odor which is not often found in the safe water.

Corrals, outhouses, and cesspools may at times be in the neighborhood of wells or springs, and one may wish to know if they drain into the well. A few simple tests will often answer. Coal oil spread over the suspected area and then washed down with water or rain will appear in the well or spring water if there is drainage. This may be detected by the odor or the fine film which forms on the surface of the undisturbed water. Similar evidence may be obtained by the use of large quantities of salt placed in the suspected area and then tested for in the water.

More accurate information may be obtained by dissolving one gram of fluorescein in a little alkali, mixing with a quart of water, and flushing into the suspected place with large quantities of water. If there is a connection between the suspected polluted area and the well or spring, the fluorescein appears in the water to which it imparts a yellowish, fluorescent color so characteristic that one cannot mistake it. This test is very sensitive since one grain will impart a visible fluorescence to over 500 gallons of water. Moreover, if traces of it are left in the water after the test it is usually harmless.

From this it can be seen that the water of an unpolluted well or spring usually comes from deep in the earth, has the same temperature summer and winter, does not become turbid after a storm, and is not influenced by coal oil, fluorescein, or other substances placed in polluted areas.

Microörganisms in Water.—Water receives its microflora from air, soil, and from the living and lifeless bodies of plants and

animals. Consequently, almost any germ, pathogen, or saprophyte may occasionally be found in water. Many of these microörganisms soon perish, some may survive for a longer period, whereas, a few will find the water a suitable permanent habitat. It is the organisms which make water their home that constitute the natural microflora of water.

The bacterial content of water varies greatly with the source, season of the year, and especially with the kind and quantity of pollution which it has received. Rain and snow water may vary from a few or even none to thousands per cubic centimeter, depending upon the nature of the atmosphere through which it has fallen and the surface on which it is caught. Deep springs and wells contain few or may be entirely free from bacteria. On the other hand, surface wells and springs contain thousands or even millions. Running streams usually contain more bacteria than standing water, but even in the case of these there is a wide variation, depending upon the turbidity and especially the sewage pollution which they have received.

Some sanitarians of wide experience are inclined to hold that natural waters which are found by approved methods to contain more than 1,000 bacteria per cubic centimeter should be considered suspicious. However, numbers cannot be taken as the sole criterion of purity, as a turbid stream free from pollution may carry many times this number and yet could not be pronounced dangerous. The number of bacteria found in a water by the various methods must always be interpreted in the light of the chemical and further bacteriological analysis, and the sanitary survey. The specific value of the bacterial count rests on the information which it imparts on a water which has been studied over a period of time for a distinct increase in numbers indicates pollution. Then too, the bacterial count made before and after purification gives a fairly accurate measure of the effectiveness of the method used.

Inasmuch as counts do not give an exact measure of the purity of the water, and it has been found impracticable and usually impossible to isolate the pathogens from them, other methods have had to be devised to determine the purity of a water. The chemical methods determine the quality and kind of nitrogen-carrying compounds in the water. From these determinations one may draw conclusions as to the nature, quantity, and quality of pollution which has entered the water, and what is more important, the probable time which has elapsed since it entered.

The most valuable and widely used bacteriological procedure is to determine the presence or absence of *Bacterium coli* in the water. This organism is an inhabitant of the human intestines, and, hence, is found in great numbers in sewage. Its presence in appreciable numbers is therefore, presumptive evidence that sewage is entering the water and along with it pathogens may find their way.

Water and Disease.—Even as early as 400 years before the beginning of the Christian era it was pointed out by some writers that there was a direct relationship between polluted waters and disease; some went so far as to advise the boiling or filtering of the water before it was used. In the centuries which followed, there was noted a direct relationship between drinking water and the great epidemics which swept over Europe. During the Dark Ages it was considered established that water was often the cause of disease, but the real agent within the water was not suspected, for many a reputed witch lost her life for the alleged poisoning of water. The real cause was not determined until the nineteenth century, when Dr. Michel of France collected such an array of statistics that it seemed highly probable that typhoid fever was due in many cases at least to infected water. In 1880 Eberth discovered the typhoid bacillus, and in 1884 Gaffky succeeded in growing Eberth's bacillus upon laboratory media. Since that time evidence has slowly accumulated that definitely shows the causative organism of typhoid fever to be this bacillus. Furthermore, many typhoid epidemics have been traced to water, and in a few cases the causative organism has been obtained from the water.

Dysentery.—The relationship between impure water and disease is shown in the declining death rate from dysentery in the United States. The mortality from this cause in 1850 was 6.32 per one hundred thousand inhabitants; in 1860 it was 2.65; in 1870 it was 1.60; while in 1880 it was less than 1.5. During all this time the population had rapidly increased, and what is more important, it had been crowded together in great centers, thus giving greater opportunity for infection. There has been, however, much more attention paid to the water supplies of cities, resulting in a decrease in the death rate from intestinal diseases.

Typhoid.—The value of pure water is shown more clearly in another way. If one takes a 5-year average of the deaths from typhoid fever in large cities both before and after the installation

of plants for the purification of drinking water, one gets the results given in tabular form below. They are stated as yearly deaths from typhoid fever per 10,000 inhabitants:

Place	5 years before purification	5 years after purification
Hamburg	47	7
Zürich	76	10
Lawrence, Mass.	121	26
Albany, N. Y.	104	28

This shows an enormous decrease in the death rate from typhoid on the installation of a purification plant. This does not, however, tell the whole story, for eminent authorities on this subject have found that where one death from typhoid is prevented by improving the water-supply, two or three deaths from other causes are prevented. An improved water-supply not only reduces the number of deaths from typhoid fever but decreases infant mortality and the death rate from gastro-intestinal disturbances in general. This is due not only to the removal of disease-producing organisms from water, but also to the removal of the many other substances and organisms in impure water which may greatly reduce the bodily vigor of the individual using it. Any thing, or any condition which in any way reduces the bodily vitality may make the individual an easy mark for disease.

Estimates vary as to the actual percentage of typhoid cases which are referable to water infection. It is placed by various authorities at from 10 to 40 per cent of the total cases. One of the reasons for this uncertainty comes from the fact that actual proof that a typhoid epidemic is due to infected water is usually indirect, for the positive isolating of the offending organism is effected with considerable difficulty and has been accomplished in only a few cases. However, strong presumptive evidence is obtained whenever waters are proved, through the presence of *Bact. coli*, to have been infected by sewage.

However, the best possible evidence showing that a specific typhoid outbreak is due to infected water is that obtained by the epidemiologist. He has learned that water-borne typhoid has certain important characteristics:

1. They may be preceded by a period of dysentery.
2. Water-borne epidemics usually have a sharp onset. The curve rises to a peak and then rapidly declines.
3. The cases are quite evenly distributed over the city; that is, provided the city is served by a municipal supply.
4. Water outbreaks nearly always occur in the spring, fall or winter.
5. The pollution is usually nearby and the epidemic is of short duration unless there is a continuous source of new infecting material.

Diseases Due to Water.—In the past, cholera has often resulted from the drinking of water containing cholera organisms. Even today this is true in some parts of the Old World. There is a marked difference between typhoid and cholera in that typhoid bacilli probably never multiply in water and rather quickly disappear, whereas, cholera organisms will survive longer and may even multiply in polluted water.

Due to the fact that water is universally used to dispose of human excreta, it often receives tubercle bacilli and other disease-producing germs. These, too, may give rise to disease when they find their way into drinking water.

It is often stated that simple goiter is due to water; for individuals drinking certain waters develop it, whereas those drinking other waters may not. In the past, this has been attributed to hardness or organic constituents in the water, but today it is known that the lack of iodine in the water and food causes simple goiter and that when sufficient iodine is supplied simple goiter disappears.

Natural Purification of Water.—Nature is constantly conducting on a gigantic scale the purification of water. This is done by distillation, sedimentation, and oxidation including the action of light and filtration.

Distillation.—It is estimated that annually, over 86,000 cubic miles of water is evaporated from the surface of the earth. This rises as a vapor and leaves the impurities behind; it condenses and falls to the earth in the form of rain, snow, or hail, nearly free from organic and inorganic matter and totally free from pathogens.

Sedimentation and Oxidation.—Surface waters which have become polluted tend to purify themselves. This is due to physical, chemical, and biological processes which are constantly at work. Suspended particles having a specific gravity greater than water tend to settle to the bottom. These carry microörganisms with them. Sedimentation is small in running streams but very great in standing water. It is probably of

first importance in lakes, ponds, and reservoirs, and is the principle reason why these waters contain fewer bacteria than running streams.

Oxidation and reduction of organic and inorganic constituents of the water with the production of simple substances which are not well suited to the maintenance of life and growth of many forms of bacteria are constantly occurring. They probably play a greater part in running than in standing water. Sunlight which plays an important but unmeasurable part in freeing waters from microörganisms is probably more effective in standing than in running water.

Furthermore, there are certain biological factors constantly at work which tend to free water of its organic material and its bacterial content. Many organisms find water an unsuited medium for their growth and die. Still others may find within the water certain factors which actively reduce their numbers. These conditions which are not well understood, are known as antibiosis or symbiosis with probably, in some places, the bacteriophage playing a predominating rôle.

Filtration.—Surface water passes into the earth to reappear in wells and springs. In loose, gravelly, and limestone formation, this may be ineffective, whereas under appropriate conditions on passing water through a few feet of fine grained soil it may have its bacterial content reduced from 95 to 99 per cent.

Whipple states: "Sandy soil is a good filtering material, and when a well in such a soil stands at the center of a circle twenty five or fifty feet in a radius in which there are no privies, cesspools, sink wastes, or other sources of contamination, the water can usually be depended upon as fit for domestic use, provided of course, that the top of the well is properly guarded against surface wash."

Artificial Purification.—Those methods which are so effective in the purification of water under natural conditions are the methods usually made use of in purifying it artificially. Only a few of the best known artificial methods can be briefly considered here.

The slow sand filter frees water from impurities by sedimentation, filtration, and the biological destruction of organic matter and bacteria. It has been extensively used for over one hundred years, the greatest impetus being given to this measure in 1893 when Koch showed that the proper filtration of the water from the Elbe River saved Altona from the epidemic of

cholera that devastated Hamburg which was using unfiltered water.

The sand filtration method consists in causing water to pass through a layer of sand of such fineness and thickness that the requisite removal of suspended substances is accomplished. The filter as usually constructed is a basin having a watertight concrete base on the surface of which are laid perforated tiles or pipes. These tiles are covered with about a foot of gravel graded in size from 25 to 3 mm. in diameter from bottom to

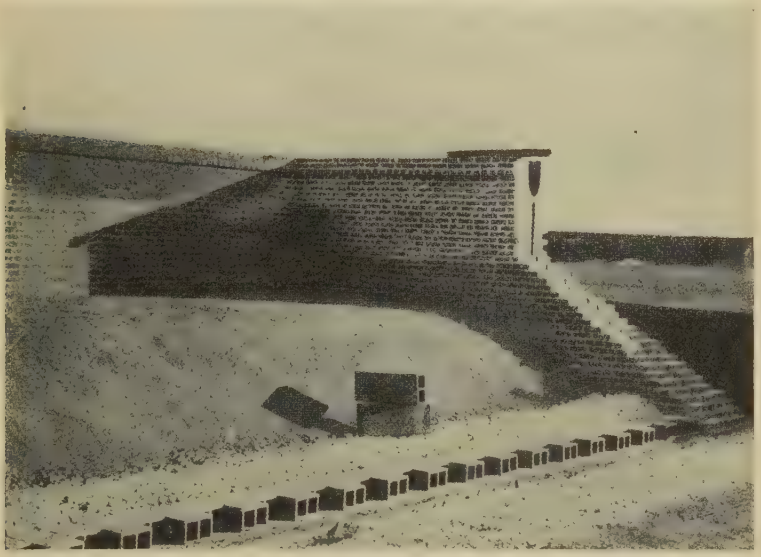


FIG. 65.—Cross-section of a typical slow sand filter. (*From Mason's Water Supply, John Wiley and Sons.*)

top. Over this is placed three or four feet of sand which acts as the real filter. The water passes through this and is conveyed to the mains by the underlying pipes. The suspended material, including bacteria, is removed by the sand which becomes more efficient as used, due to the rapid formation of a mat of finely divided sediment, in which protozoa often multiply, and assist biologically in removing many bacteria. In time the mat becomes very thick and filtration, although effective, is unduly slow. The water is then allowed to subside below the surface and about half an inch of the sand removed, after which filtration is resumed. The sand removed is washed to free it from

collected impurities and later is replaced on the bed after successive scrapings have reduced the filter to about one foot in thickness.

The filters are usually divided into units of convenient size, about half an acre, so that one unit may be cleaned without interruption of the system. The slow sand filter removes about 99 per cent of the bacteria, about one-third of the coloring matter, and its long effective use has established the fact that it has a

FIG. 66.—Filter bed No. 6 Indianapolis Water Company. (*From Boyd's Preventive Medicine.*)

favorable effect upon the health of the community in which it is used.

Chemical Methods.—The chemical disinfection of water on a large scale is now almost exclusively effected with substances, yielding chlorine, chief of which are bleaching powder (chlorinated lime), sodium hypochlorite, and free chlorine. The action of these substances is essentially similar and dependent upon the quantity of active chlorine which they contain. They are usually added in quantities sufficient to give from 0.5 to 1 part of active chlorine per million parts of water.

The use of bleaching powder in the purification of waters is cheap, reliable, harmless, and easy of application, which makes it an attractive method, but when used on impure waters containing organic matter it gives rise to amines, chloramines and other compounds of unknown composition which impart to the water unpleasant flavors.

Alum is often used either alone or in connection with the mechanical sand filter, and if used under controlled conditions is very effective and leaves no undesirable constituents in the water. The quantity should be accurately determined for each water, for the effectiveness of it varies with the turbidity and quantity of calcium carbonate contained in the water.

Potassium permanganate is often used in disinfecting small quantities of waters, but its effectiveness cannot be depended upon except against the cholera spirillum. Moreover, the disagreeable taste and the color imparted to the water are a serious drawback. Ozone and ultra-violet light are both very effective in the sterilizing of water, but expense and technical difficulties have so far prevented their general use.

Boiling can always be depended upon for the rendering of a polluted water safe and may be used temporarily to tide over a short dangerous period. The difficulties involved in this method, and the tasteless water which results from it, are great drawbacks to its more general use.

The common filters which are on the market are usually unreliable, for if fine enough to hold back the impurities their action is slow and there is a tendency for them to leak. Furthermore, they may yield a bacteria free water at first, but later the microörganisms find their way through the filter. Hence, they can be depended upon only when properly constructed and cared for by experienced hands.

CHAPTER XXI

BACTERIOLOGY OF SEWAGE

The teachings of Pettenkofer during the latter half of the nineteenth century directed the attention of health workers to sewage as a cause of disease. He taught that fecal matter undergoes a ripening process in the earth and, hence, becomes the cause of typhoid fever. He did not consider the causative agent to be alive, but thought that due to chemical reactions there resulted noxious substances. These he considered may be dissolved in the water or even disseminated in the air and give rise to epidemics. Due to his teaching, cities and municipalities started to provide for the adequate disposal of their sewage. Immediately, there resulted a decrease in the gastro-intestinal diseases. Pettenkofer's theory was wrong, but as we know today it removed the causative organisms of disease which breeds primarily in the body of man and leaves in his excretions. Since then man has learned that for both esthetic and sanitary reasons, sewage must be properly handled.

Composition.—Sewage is a variable mixture. It has very aptly been defined as the water-supply of a town or city after it has been used. It carries human and animal excreta, refuse from the kitchen, laundry, and manufacturing establishments. In cities having only a single system it may also carry huge quantities of dirt and refuse which come from the storms and flushing of the streets. Its quantity is directly proportional to the consumption of water in the districts. In small cities it may be as low as forty or fifty gallons per capita daily, whereas in larger cities it may reach from 100 to 200 gallons or over.

The composition of sewage depends upon the density of population, the number and kinds of manufacturing establishments, and whether or not there is a separate or a combined system. Where the combined system is used, the composition and quantity of the sewage varies with the rainfall and the street washing. There is usually a diminution in quantity and change in composition at night. From the viewpoint of purification,

sewage contains urea, various proteins (albumin, casein, fibrin), carbohydrates (sugars, starches and celluloses), fats, soaps, and other organic constituents. The decomposition of the proteins yield ill-smelling, offensive, nitrogen and sulfur-carrying compounds. Fats are only slowly decomposed by bacteria and in the early stage give rise to glycerol and fatty acids. The fatty acids react with calcium and magnesium with the formation of insoluble soaps, which collect on some solid moving substance such as a match, nail or other insoluble material. The water rolls them along, which often results in the formation of huge, insoluble balls that may become large enough to obstruct the flow of the sewage. Mixed in this heterogeneous mass are swarming hosts of microörganisms most of which are harmless. They soon engage in the beneficient work of destroying the organic matter. Along with these may be bacteria from persons sick with typhoid fever, dysentery, cholera, tuberculosis, and other diseases; hence, "sewage is obnoxious to the senses because of its decomposing organic matter, but it is dangerous to health because of the possible presence of pathogenic bacteria."

Bacteria in Sewage.—The number and kind of bacteria in sewage varies widely and depends upon the composition and origin. Usually there are more present in European than in American sewage. There are also more in the day than in the night. However, this may be reversed in manufacturing centers where water from manufacturing plants finds its way into the sewers. The numbers of bacteria range from 500,000 to 12,000,000 per cubic centimeter. Fuller estimates that ordinary sewage contains about 322 billion per day for each person connected with the sewer.

Sewage may or may not contain pathogens, but millions of saprophytes are at all times present. Of the latter there are both anaërobes and aërobes. The common anaërobes are *Cl. regularis, B. enteritidis sporogenes, B. amylobacter*, and *Cl. butyricum*. The aërobes are the common organisms from water, soil, air, and the human and animal intestinal tract.

Interest centers more in the changes produced by the various microörganisms found in sewage than in the specific organisms. Most of the bacteria are not only harmless, but of genuine importance in the economy of nature through the scavenger work which they accomplish. A few of them are dangerous because they may cause certain infectious diseases. Many of them play an important rôle in decomposing sewage forming malodorous

gaseous products that are associated with putrefactive nuisances. The modern tendency, therefore, is to classify sewage bacteria from a physiological standpoint. They may be classified in relation to their oxygen requirements as aërobic and anaërobic, or with regard to their action on a substance as hydrolyzing, oxidizing, and reducing. From the standpoint of sanitation it must always be kept in mind that although the majority of sewage bacteria are harmless saprophytes, that there is always the possibility of dangerous pathogens being present.

Hydrolytic Changes.—Most of the early changes which occur in sewage are hydrolytic; that is, the substance is caused to take up water, becomes unstable, and for some reason breaks into fragments. The complex insoluble compounds are broken into simpler soluble substances. This progresses rapidly under anaërobic conditions as a great variety of bacteria decompose proteins under anaërobic conditions. This process converts proteins by steps to proteoses, peptones, peptids, amino-acids, and finally to ammonia, carbon dioxide, methane, hydrogen, and so on. It corresponds in the main with ammonification; however, the changes in the septic tank partake more of putrefaction, whereas in soil decay predominates.

Cellulose fermentation, next to protein hydrolysis, is the most important work of bacteria in sewage purification. Paper, cotton fabric, wood, and other cellulose-containing substances are rapidly decomposed by various microörganisms with the production of soluble substances—starches, sugars, acids, and finally carbon dioxide, methane, and hydrogen.

Probably fewer microörganisms possess the power of saponifying fat than of liquefying proteins or hydrolyzing cellulose; and as a consequence, fat accumulates and tends to rise to the surface out of the sphere of bacterial action. At times it may accumulate around some solid and give rise to "grease balls" which cause clogging of pipes. The fat which is acted upon by bacteria is broken into fatty acids and glycerine. The fatty acids are quite resistant to further bacterial activity, whereas the glycerine is rapidly broken into simpler products.

Oxidation.—The complex microflora of the sewage must have energy. This they obtain in a great measure from the oxidation of the comparatively simple products yielded through the hydrolysis of the proteins, carbohydrates, and fats. These are caused to take up oxygen with the production of acids and finally carbon dioxide and water.

The ammonia liberated through the de-aminization of the amino-acids is oxidized by the nitrosomonas to nitrous acid and by the nitrobacter to nitric acid.

Reduction.—The nitrites and nitrates produced by the nitrifying bacteria are in a great measure rĕduced to free nitrogen through denitrification. The sulfur in the protein molecule is liberated in the form of sulfates, sulfur dioxide, and hydrogen sulfide. The sulfates formed are reduced to hydrogen sulfide. This reacts with the small amounts of iron and other metals present and forms the black residue of metallic sulfides that are always found on the bottoms of tanks and streams in which sewage is decomposing.

All of these processes go on simultaneously in sewage, for one is dependent upon the other, there being a true biological cycle, as is pointed out by Whipple: "The decomposition and oxidation of the organic matter in sewage are brought about by bacteria, and the bacteria serve as food for protozoa and other forms of microscopic animal life. The dissolved organic matter in sewage serves as food for algae. These algae and protozoa are, in turn, consumed by rotifera and crustacea, while the latter form the basis of food supply for various aquatic animals and fishes. Thus, there is a continuous biological cycle. Again, animal forms require oxygen and produce carbonic acid, while plants consume carbonic acid and produce oxygen. Where these processes occur normally and with a proper equilibrium maintained between animal and plant life, offensive conditions do not result; but where abnormal conditions are produced, as, for example, by the discharge of excessive quantities of sewage or trade wastes into a stream, a depletion of the dissolved oxygen may follow, or there may be an overproduction of algae so that the conditions become offensive. It is coming to be realized that in order to properly determine the dilution required in any particular case the conditions required to bring about this condition of biological equilibrium must be determined."

Pathogens.—Owing to the origin of sewage, as before pointed out, it may contain pathogenic bacteria at any time. The common species found are typhoid, cholera, and dysentery; however, it is possible for any of the other disease-producers to find their way into sewage, too. This is especially true of tuberculosis, the causative organism of which is quite resistant to putrefaction and may survive for some time in sewage. Most pathogens do not multiply in sewage, and some, for instance the

typhoid bacilli, seem to die more quickly in sewage than in fairly pure water. Jordan thus summarizes our present knowledge of the longevity of typhoid bacilli in sewage: "Laboratory experiments have shown that the typhoid bacillus can survive in sterile water in glass vessels for upwards of three months, and for possibly two or three weeks in unsterilized ground or surface water. Other evidence indicates that the bacillus is able to travel in water a distance of at least 140 kms., and to retain its vitality in natural bodies of water for at least four or five days. It is possible that water may continue to be the vehicle of infection during a much longer period, but the available data point to a comparatively short duration of life of the specific germ in the water of flowing streams. Under ordinary conditions no multiplication of the typhoid bacillus takes place in water, even when a considerable amount of organic matter is present, but on the contrary, a steady decline in numbers goes on. The history of typhoid epidemics tends to show that sewage pollution is to be feared chiefly when the sewage is fresh, and that the danger of infection diminishes progressively with the lapse of time.

"In soil or especially the fecal matter of privy vaults the duration of life of the typhoid bacillus is much longer than in water. Levy and Kayser found typhoid bacilli in soil that had been manured fourteen days previously with the five-months old contents of a vault. The evidence that any genuine multiplication can take place in the soil is not convincing, but it has been proved that the bacillus may be carried by water-currents to a considerable distance from the point where it was first introduced. Infection of wells and small water-courses is thus brought about sometimes by the washing of bacilli out of soil in which they may have lain dormant for many months. The persistence of typhoid fever around certain habitations may be plausibly explained on the supposition of an extensive soil infection. There is no doubt that the practice of using human excrement for manuring vegetable gardens entails a danger no less real because often unrecognized."

Methods of Disposal.—The method selected for sewage disposal will vary with the district, location, and means at the disposal of the sanitary engineer. However, in all cases he must keep in mind convenience and public health. In rural districts the well-constructed cesspool may of necessity be used, but in the urban district it must not be tolerated. One of the readiest methods, and one which until the last few years has been univer-

sally used in this country, is to allow the sewage to flow without treatment into the nearest stream, lake, or harbor. This is very successful as long as the quantity is not excessive, the dilution great, and the receiving water not used by other communities for drinking and culinary purposes. Where this method is used the dilution should be great. The Chicago drainage canal was designed on the basis of 3.3 cubic feet per second for 1000 people. The efficiency of purification, however, varies with the nature of the sewage. The presence of trade wastes, especially those of an oily nature, which float on the surface, may form scums which interfere with the absorption of oxygen from the air. Rapidly flowing streams, on account of their absorption of oxygen, tend to purify themselves more rapidly than do slower ones. Cold water holds more oxygen than warm water, and fresh water more than salt water; hence, there is a greater tendency for oxidation in cold fresh waters than in warm or salty waters.

There is, however, a growing demand that sewage be treated before it is thrown into streams or lakes. This may be done by various methods, such as sedimentation, sub-surface irrigation, broad irrigation, and other means.

Methods of Treatment.—The methods used in the treatment of sewage depend upon the predominating changes occurring in the sewage and may be divided roughly into three classes: physical, biological, and chemical.

The changes occurring in screening and sedimentation are primarily physical. When screens or strainers are used, they may be very simple metal rods or screens, or elaborate revolving cylinders covered with wire mesh. They are placed directly over the outlet of the sewer. In this manner the coarser materials which may interfere with later processes of purification are removed. In America these strainers are not used generally, for they remove only about 10 per cent of the suspended particles in American sewage.

Sedimentation is used much more extensively. This removes some 50 per cent of the suspended material, and depends on reducing the flow of the sewage which allows the coarser material to settle out. This may be accomplished by running into huge chambers where water currents are reduced to a minimum, or by filling a tank and then diverting it to a second tank while settling is occurring in the first. The coarse material which settles out is known as "sludge."

The sewage may empty directly or from the sedimentation tank into a septic tank in which it is retained for a considerable

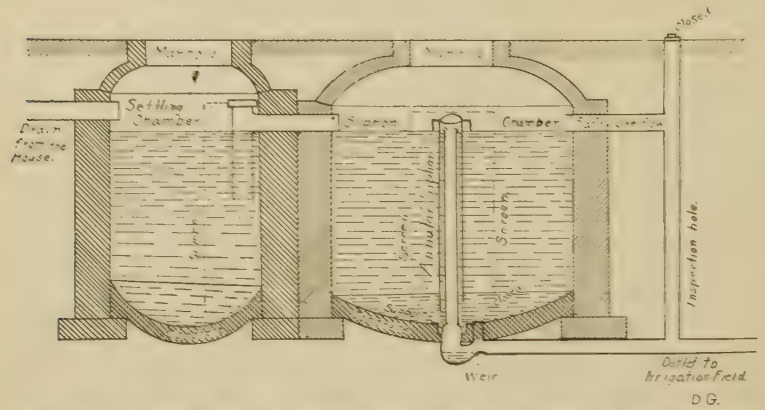


FIG. 67.—Settling chamber and fresh tank for surface and subsurface irrigation of sewage. (*Redrawn from Gerleard's "The Disposal of Household Wastes."*)

FIG. 68.—Trickling filters, Columbus, Ohio. (*Am. Mus. Nat. History Guide Leaflet No.* 33.)

time during which bacteria decompose much of the contents. During decomposition there results a film on the surface. This

shuts out the air and favors rapid anaërobic decomposition. To prevent the gases from causing the mixing of this scum two story or Imhoff tanks are used. These are so constructed that the gases may leave and the sewage enter without disturbing the scum. The sewage from the septic tank may be conveyed to sand beds, trickling filters, sewage farms, or in other ways accelerate the biological processes so as to speed up aërobic decomposition and to completely oxidize the obnoxious substances which would otherwise remain. Often any one or a combination of the above processes are followed by a disinfection of the liquid portion of the sewage. This frees it of all pathogens. In the past chlorine has been quite generally used but within more recent years electrolysis has been found to have many advantages over simple chemical treatment.

Sludge.—In the sedimentation basins, in the septic and Imhoff tanks, in the contact and aëration bed, and after electrolytic and chemical treatment, there results a watery accumulation of organic matter, the disposal of which is of economic and sanitary interest. For generations economists have recognized the enormous quantities of fat and fertilizer contained in and wasted in sewage. Hence, numerous attempts have been made to recover part and in this manner reduce the cost of sewage disposal. Up to date this has not met with anything but partial success. However, the two most promising methods are the activated sludge method and the Miles process.

In the activated sludge method raw sewage is run into aërating tanks in which air is pumped through the sewage by an air compressor. The air enters through perforated plates or pipes at the bottom of the tank. The sewage is then run into settling tanks and the sludge is collected. The resulting sludge is nearly free from odor and contains great numbers of saprophytic bacteria and is used as a fertilizer.

In the Miles process the organic material is precipitated and the fats hydrolyzed by means of sulfuric acid or in some cases, sulfur dioxide. The process removes from 61 to 66 per cent of the total suspended material and 99 per cent of the bacteria. The fats must then be separated and the products dewatered and dried. Up to the present time these last steps have not been economically successful.

What Should Be Accomplished in Sewage Disposal.—The sanitary engineer attempts to dispose of sewage as rapidly as possible, with the least nuisance to the smallest number of people,

with the least damage to health or property, and at the smallest cost. Sewage can be made entirely harmless only by the complete destruction of its organic matter and bacteria. A complete purification is not attempted normally as the plant required for such would be very elaborate and too expensive. Moreover, practical experience has shown this to be unnecessary. What should be accomplished is simply a destruction of the disease-producing bacteria, the elimination of offensive odors, the destruction of solid organic matter, and the disposal of the liquid portion in such a way as to best aid natural factors in purification.

CHAPTER XXII

MILK

Milk plays a double rôle in human welfare; that of a Dr. Jekyll and a Mr. Hyde. It is man's best food. It is all but indispensible to the growing child. It is the only food for which there is no effective substitute. It is also an excellent culture medium for bacteria, consequently, easily spoils. Due to its origin, method of handling, and nature, it probably causes more sickness and death than all other foods combined. Consequently, the principle underlying its production and consumption should be: increase the production and consumption of milk, but see that it is good, clean, and safe.

Consumption of Milk.—The total milk production in the United States in 1924 was approximately thirteen and one-half billion gallons. This would be sufficient to make a lake over three miles square and twenty feet deep. About 45 per cent of this was used as milk, and the remainder in the manufacture of butter and cheese. The average daily per capita consumption was thus slightly greater than 0.6 pint. Denmark, Norway, Sweden, and Germany all use over one pint per capita daily, whereas China, Japan, and the United Kingdom fall below the United States. In the United States the largest quantities are used in the eastern and western parts, whereas the southern part falls considerably below the average. More milk should be used, as is pointed out by Emerson, the public health authority.

"Present information as to cost and value makes it quite clear that the entire community would save expense and serve their nutritional needs if as much as one quart of whole milk were used as food for each member of the population daily. We can go further and say that it is indispensable for steady growth and development of children at least until they reach school age that a quart of milk should enter their daily dietary. We can add a third summary of the situation to the effect that unless at least a pint of milk a day per person is used in a community a waste of income and serious nutritional errors are certain to develop and effect the vitality, sturdiness and capacity to resist disease of a large

number of both children and adults. Let me add further that none of these statements imply that these are measures of what each person in the community is supposed to drink daily in the form of whole fluid milk. The use of milk in all forms of foodstuffs, soups, sauces, puddings, ices, hot and cold drinks, etc., is included in the daily designated allowance.

"This estimate of advantageous use of milk does not include the milk from which the finished products, butter and cheese, are made, nor does it include the skim milk powders, or the condensed and evaporated milk preparations.

"The above statements of an optimum per capita use of milk of one quart per day for the entire population and a minimum safe allowance of one quart for each child under six years of age and not less than one pint, for the remainder of the population is not based upon any single social, economic, chemical, or physiological test, study, or experiment, but represents the sum of observation in many relief agencies, budgetary studies of families under nursing and dietarian care, taken together with the experience of physicians and of medical surveys in hospitals, and the exact records of camps, institutions for children and adults, etc."

Value of Milk.—When milk is evaluated from the viewpoint of cost, digestibility, and nutritive value, it leads all foods. If cost is considered from energy values alone, milk is found even at present prices to be one of the cheapest foods. It is usually stated that one quart of milk is about equal in food value to anyone of the following:

2 pounds salt cod fish	¾ pound lean round beef
3 pounds fresh fish	⅓ pound cheese
2 pounds chicken	2 pounds potatoes
4 pounds beets	6 pounds spinach
5 pounds turnips	7 pounds lettuce
⅙ pound butter	4 pounds cabbage
⅓ pound wheat flour	8 eggs

This comparison is based on the energy content of the food and is only one, and at times the less vital rôle played by a food. In so far as energy is concerned the animal may use fats, carbohydrates, and proteins from different sources and interchangeably with nearly equal efficiency. However, this is not the case where food is used as a builder of human tissue. The individual breaks

the proteins into their specific amino-acids; these are sorted over and built into the tissue proteins; hence, the quantity of tissue that can be produced from a unit weight of protein is governed by the number and quantity of amino-acids contained in the food protein. Judged from this standpoint, the proteins of milk are of a good nutritive quality and are superior to the proteins of most other foods, because, milk and the leafy vegetables are the only foods so constituted as to make good the deficiencies of cereal grains, legume seeds, tubers, roots, and muscle meats. For this reason, McCollum has suggested that these two classes of foods should be designated "Protective Foods" in order to give them the prominence they deserve.

The experience of recent years has taught us the importance of the little things in the nutrition and well-being of man. Limitless quantities of carbohydrates, fats, and proteins fail to keep the human thyroid functioning in the absence of minute quantities of iodine. Likewise, a balanced diet lacking in vitamins may fail to promote growth, or may even fail to maintain life. Milk contains all of the known vitamins but is not uniformly rich in all. The fats of milk are a most important source of vitamin A which is essential for growth, as well as a protection against certain eye troubles and infections.

Milk contains appreciable quantities of vitamin D which is essential in the metabolism of calcium and without which the animal develops rickets. It also contains some vitamin B and C. The quantity of some of the vitamins in milk depends upon the food received by the cow and in the case of vitamin C the treatment of the milk. Pasteurization destroys some of this vitamin. However, this cannot be urged as an objection against pasteurization as even raw milk should never be depended upon as the sole source of the vitamins. This is true especially in the case of vitamin C.

Calcium exists to the extent of several pounds in the adult body. It not only enters into the structural frame work but is a vital constituent of the body fluids and tissues. The typical American dietary with a large abundance of cereal grains of various sorts, of potatoes and meats, of sugar and fats, with a sparse inclusion of green vegetables is deficient in calcium; consequently, it is not surprising to find Sherman stating that a deficiency in calcium is one of the most outstanding defects in the American diet. There is little doubt that this is one of the main causes of malnutrition among school children. Milk

remains today as the most available source of calcium, and if it were regularly included in the diet a considerable number of the many cases of malnutrition would disappear.

Moreover, individuals who have lived to a good ripe old age have usually used milk in some of its forms. Several French laborers whose diet consisted largely of milk lived to be one hundred ten years and over. There are also authentic records of a number of individuals in the Balkans, Persia, Arabia, and in the Caucasus mountains who have reached extreme old age, and whose diet was largely milk.

Scientists have long studied the habits of these centenarians and their diet was found to contain large quantities of sour milk. Metchnikoff attributes their long life to a specific bacterium taken into the body with the sour milk, the *Lactobacillus bulgaricus*. Few today seriously subscribe to the original theories of Metchnikoff, but it has been demonstrated that the growth in the intestinal canal of the normal *Lactobacillus acidophilus* may be increased so as to make it the predominating organism by the liberal inclusion of fresh or soured milk in the diet.

Milk in one form or another can be taken by most individuals. It is a very easily digested food, in fact it is often necessary to place children and individuals whose digestive system has been impaired by faulty diet or disease on a milk diet. However, for the adult, milk is not a perfect food, because it is too bulky and is deficient in iron. That it has general far-reaching nutritive value is well understood. McCollum writes:

"Those people who have employed the leaf of the plant as their sole protective food are characterized by small stature, relatively short span of life, high infant mortality, and by contented adherence to the employment of the simple mechanical inventions of their forefathers. The people who have made liberal use of milk as a food, have, in contrast, attained greater size, greater longevity, and have been much more successful in the rearing of their young. They have been more aggressive than the non-milk-using people, and have achieved much greater advancements in literature, science, and art. They have developed in a higher degree educational and political systems which offer the greater opportunity for the individual to develop his powers. Such development has a physiological basis, and there seems every reason to believe that it is fundamentally related to nutrition." Yet surveys reveal the fact that milk, the best and most economical food, is used by the poorer classes in the smaller

quantities and what they do use is obtained in the most expensive form, condensed milk.

Classes of Milk.—It is generally recognized that there is a great difference in meat, bread, and vegetables, but it is not so generally understood that milk varies in quality and in nutritive value. All milk should be graded. Some writers say there are only two grades of milk—good and bad. There is a growing tendency to classify milk as pasteurized and raw milk. However, the more general classifications recognized are: "Certified milk," "Grade A," "Grade B," and "Grade C."

Certified milk is the very best, freshest, cleanest, and purest raw milk that it is possible to produce. It is milk of uniform composition and of high quality obtained by cleanly methods, from healthy cows under special sanitary care. It is so certified by a medical milk commission. The cows have been examined by a veterinary surgeon to find if they are free from disease, especially tuberculosis. They are kept as clean as it is possible in a properly constructed, lighted, and ventilated barn. They are fed only good clean feed and water. A physician looks after the health of the milkers, and the greatest care is taken to see that the milkers wear clean suits, have clean hands, and use clean, sterilized milking utensils. The milk must meet certain high chemical and bacteriological standards. It must be bottled and iced at the dairy and should not be over thirty-six hours old when delivered to the consumer. It costs more than ordinary milk and is intended especially for babies and invalids.

"Grade A" milk is a good grade of milk obtained from healthy cows, by clean methods. It is produced under good sanitary conditions by individuals free from diseases. It is constantly kept clean, cold, and covered, and should not contain over 10,000 bacteria per cubic centimeter when delivered to the consumer. It should come from dairies which score at least 80 on the United States Bureau of Animal Industry Score Card. However, to make sure that it is safe it should be pasteurized.

"Grade B" milk comes from cows free from disease and is produced and handled under such sanitary conditions that the bacterial count never exceeds 1,000,000 per cc. It should always be pasteurized under official supervision, and the bacterial content should not exceed 50,000 per cc. when delivered to the consumer.

"Grade C" milk should also come from healthy cows, but is not produced and handled under conditions as good as the other

grades of milk. It is strictly an inferior milk, and like Grade B should never be used until pasteurized.

Bacteria in Milk.—Milk is not only one of the best foods for man but it is also an excellent food for bacteria, as is seen from the facts that millions are often found in a few drops. In many cases the bacteriologist finds it one of the best media in which to grow his laboratory cultures. Milk should, thus, be produced and handled so as to keep bacteria as much as possible from entering. This is especially true concerning the pathogens. Large numbers of bacteria in milk indicate dirt, lack of refrigeration, or age. Such milk may or may not contain the germs of disease, but there is the possibility of it containing them. Hence, milk with a high bacterial content is not necessarily harmful, but when used as a food—particularly for children—is a hazard too great to be countenanced, or as stated by Conn: "Good, clean, fresh milk will have a low bacterial count, and a high bacterial count means dirt, age, disease, or temperature. A high bacterial count is, therefore, a danger signal and justifies the health officer in putting a source with a persistently high bacterial count among the class of unwholesome milk."

The number of bacteria occurring in milk varies with age, initial contamination, the care with which it is handled and kept, and temperature. Milk may contain a few or millions in each drop, or some market milks at times contain as many, but not as dangerous, microörganisms as sewage.

The number of bacteria in milk is determined either directly or indirectly. Under appropriate conditions direct counts may be made of the bacterial content of milk or by a less direct method the numbers may be determined by plating on a suitable medium and later counting the number of colonies which have developed. Each have their advantages and disadvantages and give only an approximation of the number actually occurring in milk.

The bacteria and especially the keeping qualities of the milk can be quite accurately gauged by the methylene-blue test. This is based on the fact that the color imparted to milk by the addition of the dye, methylene blue, disappears more or less quickly, the time required for its disappearance depending on a number of factors, the most important of which are the bacterial content of the milk and the temperature at which the test is made. If all controlling factors are kept constant except the bacterial content of the milk, the time required for the color to disappear varies with the number of bacteria.

Due to the inexactness of the information yielded by counts alone, some workers have searched for specific groups of bacteria in milk. The colon group has long been considered an important measure of sanitation as the members occur in the alimentary canal of animals. However, recent work makes it questionable whether great numbers of *Bact. coli* are an accurate indication of sanitary milk production or simply age and improper refrigeration. They, like the actinomyces which get into milk from dust, hay, and straw, are objectionable because of their action on the milk and milk products. The presence of the colon group in milk used for making cheese gives rise to a product inferior in texture and flavor, whereas actinomyces may under certain conditions impart an obnoxious bitter-moldy taste to milk.

How Bacteria Get in Milk.—The bacteria that are present in the milk which reaches the consumer have come from two distinct sources: (1) The seeding of germs which the milk receives in its production and journey to the consumer; (2) the growth which takes place after the germs are seeded into the milk.

Milk as it is secreted by the healthy cow is a sterile fluid, but as drawn from the udder it is not. It may contain as few as 25 microörganisms per c.c. or the number may reach the thousands. Some cows give milk with few bacteria, others with many. Bacteria find their way through the orifice of the teat and, hence, are more numerous in the first milk than in the other milk. They are usually cocci and from the healthy cow are all nonpathogens.

During the milking process more germs are introduced. These come from (*a*) the coat, udder, and teats of the cow; (*b*) from the milking shed and clothes of the milker; and (*c*) from the hands of the milker. It is impossible to produce clean milk from cows that are so covered with filth that one cannot distinguish the color of their coats a few rods away. Even where the animal is in a fairly clean condition the wiping of the udder just before milking greatly reduces the number of bacteria in the milk. An average of thirteen experiments at the Storrs Connecticut Experiment Station yielded the following results:

	Bacteria in milk per c.c.
Unwiped udders	7,048
Wiped udders	716
Decrease due to wiping	6,342

Numerous investigators have shown the presence of large numbers of bacteria in cowsheds, and many individuals have seen stables or milk houses in which each beam of light passing through the crevices seems to be filled with myriads of dancing specks. These dust particles carry bacteria and if they find their way into the milk will increase its bacterial content.

Then, too, the hands of the milker may not be quite clean, and perchance have come in contact with disease germs from his

FIG. 69.—Sediment disks showing from 0.01 to 0.5 grams manure removed from different bottles of milk. (*Bull. 642 U. S. Dept. of Agr.*)

own or someone else's body. These bacteria may find their way into the milk, where they sometimes multiply with enormous rapidity.

The extent to which the milker influences the bacterial content of milk is clearly illustrated by the following experiment reported by Stocking. The average of nineteen tests with two milkers, one who had had no training in dairy sanitation and one who had, showed 17,105 bacteria per c.c. for the untrained man and 2455 for the trained man. The only difference was the knowledge possessed by the trained man.

Until a few years ago the barn and its surroundings were considered the main factors in governing the number of bacteria in milk. This feeling found expression quite frequently in summary orders from health departments to milk producers asking them to either provide better barns, or quit furnishing milk for the municipal supply. However, recent investigations have shown that in the matter of milk contamination the utensils used are of much greater importance than the surroundings. Many buckets are wrongly constructed or improperly cleaned; hence, every seam contains hidden away millions of bacteria. These, on reaching the fresh, warm milk, immediately grow. Furthermore, the strainer may contain a goodly seeding of bacteria. It would, indeed, be a great step forward if the strainer could be done away with, for then greater care would have to be taken in the production of milk, in order to have the product fit to sell. The present condition is somewhat similar to that which existed when it was first suggested that bread be wrapped. At a bakers' convention the subject of wrapping bread had come up for consideration and the members had practically agreed that all bread offered by them should be wrapped, when an old veteran arose and said: "If we wrap our bread in white paper and handle it as we do now the paper will be so dirty when it reaches the consumer that he will refuse to buy." So it is with milk; if it had to be sold in the condition in which it comes at times from the barn, it would be refused. Not that the strainer reduces the number of bacteria in the milk, for it does not; it only removes the particles which are visible to the naked eye after they have been washed nearly free from bacteria.

Purcha and co-workers studied the influence of the utensils that normally came in contact with the milk both at the barn and at the dairy. They found that at least 80 per cent of the germ life that gets into milk comes ordinarily from the utensils in which the milk is handled. The milk can, the clarifier, and the bottle filler, when improperly cleaned and handled, were all prolific sources of contamination. Thus, while milk should be produced in clean, well-lighted barns, it is possible to produce a good grade of milk in poor barns; but it is impossible to produce good milk in improperly constructed and unclean utensils.

The contamination in transit and on the dealers' premises varies widely. Milk bottled and sealed at the dairy or municipal bottling plant can be delivered without any new seeding; but if it is pasteurized and then bottled or sold as "dip" milk from

large containers, the additional seeding is often prolific. Milk should be sealed and pasteurized in the final container, and if the bottle is not covered with a cap fitting well over the neck of the bottle the top may be flamed for an instant before removing the cap. This will destroy any germs adhering to the neck of the bottle and is a worthwhile precaution, especially during epidemics, for it prevents the seeding of the milk as it is poured from the bottle.

How to Produce Clean Milk.—By clean milk is understood milk of good flavor, from healthy cows, free from dirt and containing only a small number of bacteria, none of which are harmful. The production of such milk depends upon (1) clean healthy cows, (2) the use of proper milk pails, (3) the handling by

FIG. 70.—Types of milk pails. Narrow top pails are best. (*Bull.* 56 *Hygienic Laboratory.*)

people free from disease, (4) the properly cleaning and sterilizing and drying of all utensils and containers which come in contact with the milk, (5) immediate and proper cooling, (6) storage at a low temperature, (7) keeping away from abnormal flavors and odors, and (8) prompt delivery.

Clean Healthy Cows.—The cows should appear well and show no udder trouble. The milk of cows suffering with garget should not be used. Cows should be tested yearly for tuberculosis, and those that react should be removed and the remainder of the herd tested twice a year until the herd is entirely free. Cows should be kept in clean barns and pastures. They should be kept clean. The use of a brush and curry comb each day is often necessary to keep the cows in a clean condition. The clipping

of the hair from the udder and flanks is a great aid, for this prevents dirt from sticking to them. A clean damp cloth should be used to remove dust and hair from the udder before milking.

Milk Pails.—Milk pails should be seamless and partly covered so as to prevent dirt from falling in from the body of the cows. The buckets should be biologically clean.

Milkers.—Milk should be handled only by healthy people. Individuals who are indisposed, suffering with sore throat, or who have been in contact with the sick should not handle milk as

FIG. 71.—Cleaning cows preparatory to milking. (*Bull.* 56 *Hygienic Laboratory.*)

some of the pathogens, especially typhoid, readily multiply in milk and many of them may be carried in milk from the sick or healthy carriers to well individuals.

Clean and Sterilized Utensils.—Every container and utensil from the milking pail to the delivery bottle adds a few or many microörganisms, depending upon its bacterial content. Recent work has shown that this is in many cases the most prolific source of bacteria. Hence, milking utensils should be: (1) properly cleaned with hot water; (2) sterilized with washing soda, live steam, or dry heat; (3) if soda be used this should be thoroughly

rinsed from the bucket; and (4) except where dry heat has been used, the utensils should be carefully dried, which is done best with dry heat.

Cooling.—Milk should be quickly cooled to 50° F. or below. This can be done only by placing in cold running water or ice. The placing of large quantities of warm milk in a tub of cold water is not sufficient to properly cool the milk. It may be

FIG. 72.—Clean surroundings, clean cows, milkers dressed in proper suits all help in the production of clean milk. (*Bull.* 56 *Hygienic Laboratory.*)

stirred occasionally, so as to insure uniform cooling throughout but care must be exercised to see that dust and dirt are not added.

Storage and Delivery.—The milk should be stored at 50° F. or below and kept at this temperature during delivery. It should be delivered in closed vessels.

Growth of Bacteria in Milk.—Saprophytic and many pathogenic bacteria multiply in milk so that the number found in it is governed, in addition to the factors considered above, by age and

temperature. The influence of temperature is illustrated by the following:

Temperature maintained for 12 hours	Bacteria per c.c. at end of 12 hours	Hours to curdle at 70° F.
40	4,000	75
45	9,000	75
50	18,000	72
55	38,000	49
60	453,000	43
70	8,800,000	32
80	55,300,000	28

All of these samples at first contained the same number of bacteria but were kept for twelve hours at the different temperatures and then all maintained at the high temperature. At the end of twelve hours there were over ten thousand times as many bacteria in the sample kept at a high temperature as in the one

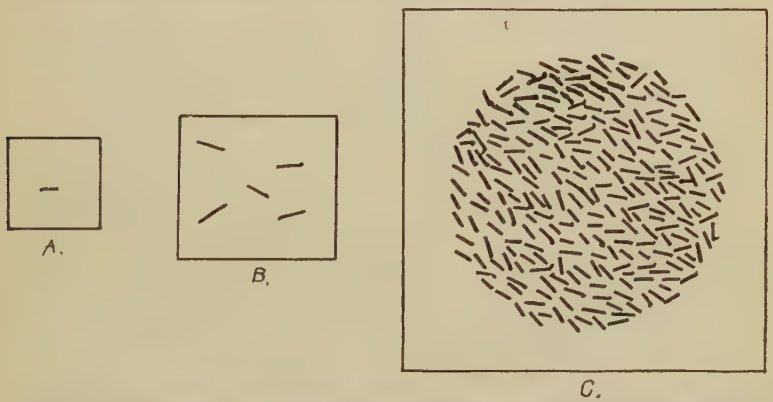


FIG. 73.—Diagram showing the effect of temperature on the rapidity of bacterial growth in milk. A, Original germ; B, growth in 24 hours when milk is properly cooled to 50° F. or below; C, growth in 24 hours when milk is not properly cooled.

kept at a low. Although the difference in temperature was maintained for only twelve hours, the milk at 40° F. kept three times as long as did that held at 80° F.

Where milk is not properly iced during delivery there may be, and usually is, an increase of over 500 per cent in the germ content above what it was when it left the pasteurizing plant.

Classes of Bacteria in Milk.—Milk obtains its microflora from various sources; hence, as is to be expected, it carries a heterogeneous lot which have been variously classified by different workers. Hasting roughly divides microörganisms found in milk into five great classes: (1) Acid-forming bacteria; (2) peptonizing bacteria; (3) bacteria producing milk of unusual color; (4) inert microörganisms, and (5) pathogenic bacteria.

Ayers and Johnson have grouped the saprophytic bacteria found in milk into five groups:

1. Acid-coagulating—those bacteria which produce sufficient acid to coagulate the casein promptly.
2. Acid-noncoagulating—those which produce acid, but do not coagulate the milk.
3. Inert bacteria—those which produce no visible change in litmus milk in two weeks.
4. Alkali-forming bacteria—those which produce an alkaline reaction in milk.
5. Peptonizing bacteria—those which liquefy the proteins of milk.

Normal Changes Produced in Milk by Bacteria.—Fresh normal milk has an amphoteric reaction when tested by means of litmus. When titrated with phenolphthalein as the indicator, it has an acidity which varies from 0.10 to 0.21 per cent. The acidity increases on standing, the rapidity depending upon the bacterial content and the temperature at which it is held. The increase is very slow at first. This is associated with the so-called "bactericidal phase," during which numerous germs which have found their way into the milk are destroyed, or at least agglutinated. When the milk has reached an acidity of 0.3 per cent it curdles on boiling, and somewhere between 0.60 and 0.86 per cent it coagulates spontaneously. The production of acid may continue until the acidity of the milk has reached 1.25 per cent. On reaching the maximum acidity, which varies with the temperature at which the milk is held, the acidity gradually decreases. After a time the milk becomes neutral and eventually alkaline.

During this series of changes first one type of bacteria and then another predominates, there being a sort of rotation of minute plants in the milk. At times milk curdles and is still sweet; this comes from the growth within it of rennet producing microörganisms that attack the casein. The hay bacillus, as well as some of the micrococci may give rise to this condition.

Lactic Acid and Bacteria.—The bacteria which produce lactic acid in milk have been variously classified by different authors. Jensen divided them into three groups: (1) Those that grow at 25° to 50° C. To this group belong the long bacilli. (2) Those that grow at a wide range of temperature; namely, from 5°–7° C. to 45°–50° C. These are all streptococci. (3) The genuine lactic acid bacteria that grow well at a temperature ranging from 10° to 40° C. This group does not include the coli-aërogenes group.

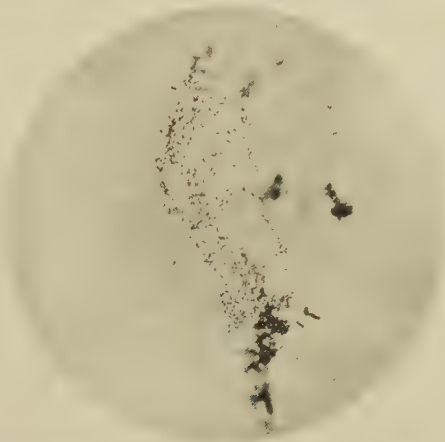


FIG. 74.—Microscopic appearance of milk that has been handled in poorly cleansed milk cans. This picture shows a film of short, rod-shaped bacteria washed from the moist surface of the metal. Originally the film probably was much larger than this but was broken into pieces when the can was filled with milk. Even so, it probably would not break up completely into its component parts in preparing dilutions for agar plates. The species of this type of bacteria cannot be recognized by microscopic examination alone. ×600. (*After Breed.*)

Löhnis proposes a more elaborate classification. He divides lactic acid producers into five groups according to their efficiency in producing lactic acid.

"Generally, the smallest quantities of acid are produced by the micrococci; next in activity come the intestinal lactic acid bacteria; stronger and much purer is the lactic acid formation by the streptococci; and the largest quantities are usually produced by the lactobacilli, although a comparatively long time is required before the maximum is reached. The usual maximum for the streptococci is from 0.5 to 0.8 per cent lactic acid and for lactobacilli fron 1 to 2 and sometimes 3 per cent. Naturally, this

general classification does not fit every individual case; exceptionally strong and weak varieties may be found in every group.

"Whether one or the other group predominates in milk or in dairy products depends on the mode of infection, the temperature, the presence of air, and the composition of the substrate.

"Concerning the mode of infection, it is to be pointed out that the micrococci come mostly from the udder, from the air, and from the utensils; the intestinal lactic acid bacteria, from feces,

FIG. 75.—Microscopic appearance of milk, 16 hours old, souring normally. The predominant organism is *streptococcus lactis* Löhnis. This occurs normally in milk as pairs, threes, or double pairs of lance-shaped cocci. Milk of this type is usually produced through improper cooling, and it is usually at least 12 to 16 hours old when examined. Less commonly, the same condition is produced immediately on filling a can that has previously contained sour skimmilk or similar material. ×600.

The small insert shows two pairs of *streptococcus lactis* ×2,000 taken from this preparation. One pair shows faint indication of division into four cells. (*After Breed.*)

from impure water, and from unclean vessels; the streptococci mostly from containers, more rarely from the cow (udder, hair, etc.); the lactobacilli from silage and other food, saliva, feces, soil, and cheese. If the temperature is kept below 10° C. micrococci will predominate after a few days; around 20° C. streptococci are very active; 30° to 40° C. are most suitable for the majority of the intestinal lactic acid bacteria; and at 45° to 50° C. hardly anything but lactobacilli survive. This general classification is again not without exceptions, but it is well worth

knowing that around 20° and 45° C., the cleanest and most rapid formation of lactic acid bacteria is to be expected, while below 10° C., and between 30° to 40° C., many by-products are formed which act unfavorably upon taste and flavor. If milk cultures of lactic acid bacteria are kept continually at high temperatures, practically all of them are killed after a few days, while at 0° C. most cultures will remain alive for a long time, especially if enough chalk is added to neutralize the acid formed. The presence of air favors micrococci and intestinal bacteria, while the majority of streptococci and lactobacilli grow best under anaërobic conditions."

Abnormal Changes in Milk.—At times foreign undesirable microörganisms find their way into milk and produce abnormal, objectionable changes.

The *Bact. coli commune* and the *Bact. lactis aërogenes* types produce considerable gas and disagreeable odors and flavors in milk and dairy products.

Bacterium lactis viscosum and related species produce a slimy or ropy condition in the milk. The slimy condition is thought to be caused by the mucin containing capsule which surrounds these bacteria. This change in milk has been known in every land and suggests that the causative organism is quite generally distributed. According to Hammer and Prucha these organisms are kept down by the normal souring of milk; but when the acid germs are destroyed there is a greater tendency for these organisms to make their presence known. However, by exercising care in the production of milk and especially in the sterilizing of utensils, the trouble caused by these undesirable bacteria may be eliminated. Slimy milk occurs more often in fall and spring, when the producer, vender, and consumer consider it unnecessary to ice milk; hence, proper refrigeration helps greatly in the control of this nuisance.

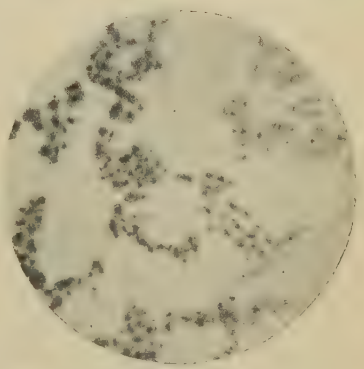


FIG. 76.—Bact. lactis mucosum. (*After Buchanan and Hammer.*)

Besides those species mentioned above, there are several other groups of bacteria that often impart bad flavors to the milk in

which they grow. For example, the actinomyces under certain conditions of storage bring about an obnoxious bitter moldy taste in milk. These organisms are numerous on straw, hay, grain, and soil, and reach the milk from these sources. The bitter flavor, bacteria cause in milk, may result from the hydrolysis of the proteins of the milk with the production of proteoses and peptones. Milk that putrifies with the production of a bitter alkaline product is likely to cause illness, if used. Many other off-odors and tastes, such as fruit-like odors, soapy milk, and the like, may occasionally occur in milk because of the presence of specific microörganisms.

Numerous bacteria possess the power of producing pigments when grown in milk; which accounts for the colored milks that one finds occasionally. A yellowish color is due to certain micrococci or short rods, a greenish discoloration to *B. fluorescens*. Blue milk may be due to *B. cyanogenus*, a pink color to *Erythrobacillus prodigiosus* a deep red to *B. erythrogenes*, and a yellow to *B. synxanthus*. Practically all of the organisms producing these various changes in milk can be considered nuisances, but they are not necessarily danger signals.

Milk and Disease.—As already mentioned, disease-producing microörganisms often find their way into milk. The principal diseases conveyed by milk in the order of importance are: tuberculosis, typhoid fever, septic sore throat, scarlet fever, and diphtheria. Occasionally other diseases such as foot-and-mouth disease, malta fever, anthrax, and indefinite digestive disturbances may result from infected milk. Recent work has shown that contagious abortion may cause illness when milk infected with the causative organisms is used by weakly children.

Typhoid fever, diphtheria, and probably scarlet fever come only from human cases or carriers. Septic sore throat and tuberculosis may be either of human or bovine origin, whereas the other named diseases come only from diseased animals.

Milk is infected in numerous ways, chief among which are the following:

(1) The pathogens may find their way into the milk from diseased animals.

(2) The milker or vender of the milk may be suffering with a communicable disease in a mild unrecognized form.

(3) A third and very important source of infection comes from carriers who work on farms, in dairies, and other places where milk is handled.

(4) A rather common source of infection is where the milker or vender of milk comes in contact with sufferers of communicable diseases while attending to his regular dairy work.

(5) Occasionally infected water is used for the washing of milk utensils, and in this manner the milk becomes infected.

The relative importance of milk in the conveyance of disease may be seen from the following table given by Kelley and Webber for Massachusetts:

Disease	1907–1915		1915–1918		1919–1923	
	Cases reported	Per cent traced to milk	Cases reported	Per cent traced to milk	Cases reported	Per cent traced to milk
Typhoid fever	23,482	9.43	6,331	7.83	4,165	7.23
Septic sore throat	.....	....	1,401	61.88	829	14.23
Scarlet fever	70,569	3.89	25,328	.55	46,777	.11
Diphtheria	69,646	0.18	33,807	.08	42,389	.018

In addition to this, many cases of tuberculosis are conveyed by milk. Rosenau estimates that approximately 7 per cent of the cases of tuberculosis in human beings are brought about by infected milk.

The principal reasons for milk conveying disease should be clearly understood. These have been summarized by Rosenau as follows: "(1) Bacteria grow well in milk; therefore, a very slight infection may produce wide-spread and serious results. (2) Of all food-stuffs, milk is the most difficult one to obtain, handle, transport, and deliver in a clean, fresh, and satisfactory condition. (3) It is the most readily decomposable of all food. (4) Finally, milk is the only standard article of diet obtained from animal sources consumed in its raw state."

Pasteurization.—The fact that pathogens may find their way into milk during its production has led many health workers to hold that there is no more excuse for the drinking of raw milk than there is for eating raw meat. Probably the former is more dangerous than the latter; but be it as it may, the fact is that there is a growing demand for pasteurization which is variously defined by different state and city laws. Generally speaking,

it simply consists of heating milk to a temperature below the boiling point and above 60° C. for a given length of time. The object of this treatment is two-fold: (1) It destroys all pathogens which may occur in milk. (2) It increases the keeping qualities of the milk.

There are two general systems of pasteurization in common use:

(1) Pasteurization with the application of a high temperature 80° to 90° C., or sometimes higher, for a period of three minutes. This is known as the flash point method.

(2) Pasteurization at a comparatively low temperature, from 60° to 65° C. for from 20 to 30 minutes, and which is known as the holding system.

Now that the main technical difficulties in the use of the holding system are being overcome, it is the one preferred. The law of the city of Baltimore dealing with pasteurization defines pasteurized milk as "milk which has been uniformily heated to a temperature between 142° F. and 150° F. and maintained at that temperature for not less than 30 minutes and cooled immediately to a temperature of 45° F., or less."

The milk when thus treated should be in closed vessels, preferably in the container in which it is to be delivered to the consumer.

A properly pasteurized milk is a safe milk in which all of the pathogens and approximately 99 per cent of the total bacteria have been destroyed. Pasteurized milk spoils as does raw milk, and if carelessly handled may be re-infected; hence, pasteurization is merely a preventive measure and should supplement and in no way replace the principles laid down for the production of a clean milk.

Evaporated Milk.—Evaporated milk consists of whole cows' milk concentrated to slightly less than one-half its original volume. It is then canned and processed under steam pressure. The finished product should be sterile; however, this is not always the case and it may spoil. There have been cases reported in which the milk became bitter but otherwise appeared normal. The bitter flavor came as a result of cleavage of the proteins by specific microörganisms.

The raw milk used in the production of evaporated milk should be of a high grade, low in acidity, and free from abnormal flavors. If the acidity is high, it coagulates on evaporating. If abnormal flavors are present they become intensified.

Sweetened Condensed Milk.—Sweetened condensed milk is concentrated whole milk to which approximately 40 per cent of cane sugar has been added. It is not sterilized, and consequently contains bacteria; but they seldom multiply in the high concentration of sugar, although some workers have found as many as a million microörganisms in a cubic centimeter of sweetened condensed milk. Most of the bacteria present are micrococci, which give rise to a thickening of the milk. Various bacilli have also been isolated from sweetened condensed milk and some of these cause abnormal flavors.

Milk Powder.—This is prepared mainly by two processes. In one, the milk is dried in films on hot rollers; in the other, it is sprayed into hot chambers and dries while in the air. In this latter case, the proteins remain soluble. Milk powder has the advantage that it may be kept over long periods, as the finished product seldom has over 2.5 per cent moisture. The drying probably destroys all pathogens but not all saprophytes. The number of these in the powder as it leaves the drier is very low, usually only a few hundred per gram. However, it may receive a liberal seeding in the handling processes. If stored for sometime, all except the more resistant spores die. The remade milk powder is highly nutritive, acceptable in taste and appearance, and in most cases can be used wherever milk is used. It is low in vitamins, and when brought back into solution spoils just as does fresh milk.

CHAPTER XXIII

MILK PRODUCTS

Slightly over one-half of the twelve billion gallons of milk produced annually in the United States is used for the production of butter, cheese, and other milk products. The nature of these products varies with the milk from which they are made, the methods used in their production, and the chemical and biological changes which occur in the finished product. The changes occurring may be desirable or undesirable and depend upon the seeding of microörganisms which the milk has received and the conditions under which the products were produced and stored. Modern bacteriological investigation has made it possible to control the nature of the germs, and to a degree the changes which they produce, and hence, to control the nature of the finished product.

BUTTER

Composition.—Butter is prepared from cream by churning and is a mixture of fats, water, milk proteins, pigments, and other products. It varies in composition, as may be seen by the following summary of some 300 analyses reported by König.

COMPOSITION OF BUTTER GIVEN AS PER CENT

	Water	Fat	Casein	Milk	Sugar	Lactic acid	Salt
Minimum.....	4.15	69.96	0.19	....	0.45	....	0.02
Maximum.....	35.15	86.15	4.78	....	1.16	....	15.08
Mean.....	13.59	84.36	0.74	0.50	....	0.12	0.66

The fat is a mixture of olein, myristin, palmitin, stearin, laurin, butyrin, and, in smaller quantities, other neutral fats. Many of these fats on exposure to air and light and especially under the influence of bacteria, yield glycerine and fatty acids. It is the fatty acids that give to old butter its rancid odor and taste. The

moisture, lactose, and casein which are mechanically held within the butter are the pabulum in which the microörganisms multiply. When the action of the microörganisms are inhibited by salt or low temperature their enzymes continue to act favorably or unfavorably on the butter.

Microörganisms in Butter.—The germ content of butter varies with a number of factors, the more important of which are:

(1) *Cream.*—Butter made from sweet, clean cream has a low bacterial content at first as compared with butter made from sour cream. The sweet cream butter lacks flavor and the yield is not as great as from sour cream. The microörganisms in the butter made from sour cream are principally lactic acid bacteria. However, it may contain many others; even pathogens have been obtained from butter made from poor-grade milk. Market milk due to its great variation yields a variable product. In the more modern dairies the cream is first pasteurized and then seeded with a culture of lactic acid bacteria. This has two advantages: (1) It destroys all pathogens which may be in the cream. (2) Pasteurization destroys about 99 per cent of the bacteria in the cream; hence, when inoculated with a reliable starter, ripening proceeds normally, and the product is of a uniform high grade. The temperature usually recommended for pasteurization is between 74° and 80° C. Temperatures below this give a product of poor-keeping properties, and at higher temperatures the flavor is affected. The ripened cream contains from one-half to three billion bacteria per cubic centimeter. The number in the butter varies with age and conditions under which it was produced and stored. Löhnis gives the following numbers in butter:

	Sweet cream butter		Sour cream butter	
	Inside	Outside	Inside	Outside
Fresh butter	1,000,000	1,000,000	10,000,000	10,000,000
Butter one week old	30,000,000	10,000,000	8,000,000	3,000,000
Butter two weeks old	2,000,000	600,000	2,000,000	100,000

(2) *Water.*—The water used in washing the utensils and rinsing the butter may carry into it many objectionable microörganisms. Water used for these purposes should be low in bacteria and above suspicion; otherwise, it should be boiled before using.

(3) *Salt.*—The salt may carry into the butter many undesirable resistant microörganisms, and if it contains small quantities of iron or copper may give rise to unfavorable flavors in the butter.

(4) *Utensils.*—The utensils used in the handling of milk are often responsible for 80 per cent of the microörganisms added to it. Many of these are undesirable. The best churns and containers are made of wood. Metals tend to impart to butter offensive flavors. All apparatus must be carefully sterilized and dried each time after using.

(5) *Paper.*—The paper used to wrap the butter may also carry undesirable germs or substances which impart to butter off-flavors. Molds are especially likely to be added in this manner. The previous boiling of the paper in a 25 per cent salt solution not only destroys the microörganisms but removes from it any objectionable flavors. As all molds are aërobic, the exclusion of air by proper wrapping prevents their growth.

Kinds of Microörganisms in Butter.—Many saprophytic and occasionally some pathogenic microörganisms occur in butter. The principal pathogen coming from the cow is the tubercle bacilli which in the past has been occasionally found in butter. This can be entirely eliminated by the use of pasteurized milk, or milk from only non-reacting tuberculin-tested cows. Typhoid bacilli may reach the milk from missed cases, the healthy carrier, or infected water. Typhoid bacilli readily multiplies in milk but tends to perish in butter. However, this organism has been obtained from butter under experimental conditions several weeks after inoculation. The longevity of *Bact. typhosum* in butter varies greatly with the temperature and composition of the butter, but the evidence is conclusive that butter should not be produced from infected milk. Pasteurization of the cream and the careful supervision of the workers is the only safeguard available in the prevention of butter infection.

The main saprophytic organisms in butter are the various lactic acid bacteria. The principal type is usually the streptococci, but the microflora varies with the organisms accidentally or intentionally seeded into the milk and cream and also the temperature at which it is stored. According to Löhnis, butter contains also quite regularly, though in small numbers, *B. fluorescens*, *B. amylobacter*, *B. subtilis*, and *B. mesentericus*. Low-grade butter is comparatively rich in *Bact. coli*, and also contains active actinomyces, yeasts, and mold. The molds multiply at

the surface of improperly wrapped butter or in a product in which considerable air has been incorporated. It has been found that in dairies producing butter with a high yeast and mold count, lax methods of pasteurization and handling of cream prevails; hence, the yeast content is quite generally used as an index of efficiency.

CHEESE

Composition.—Cheese consists mainly of casein, fat, water, small quantities of other milk constituents, salt, and often added coloring material. The nature and composition varies widely with different kinds. There are about 400 varieties which can be grouped into some 20 distinct types, as many of the varieties differ only in name and most of the names originated in some locality where the cheese was manufactured. The different varieties depend upon the nature of the milk from which the cheese was made, the methods used in its manufacture, and the conditions under which the ripening process took place. The manufacturing process can be divided into four stages: (1) The casein is coagulated into a mass of curd by means of pepsin or rennin in the presence of lactic acid produced from the milk sugar by the bacteria originally in the milk or added with the enzyme. The coagulum that results from the action of the rennet holds most of the fat and some of the milk solids. (2) By special manipulation the curd is separated from the whey. (3) The curd is salted and compressed to give it definite form and to remove the whey. (4) The cheese is kept for a period ranging from a few weeks to several months under definite conditions of temperature and moisture, depending on the variety of cheese being made. During this ripening or curing process, various changes occur because of bacterial and enzymic activities which impart to the cheese its characteristic flavor and aroma.

Microörganisms in Cheese.—It is desirable that the microörganisms in milk and butter be low, but in cheese great numbers of the proper microörganisms are essential to bring about the desirable changes. The number and kind vary with the milk from which the cheese is made, the rennet used, (home-prepared rennet is high in bacteria), the methods used in the making, the variety of cheese, and the age. That the variation is great may be seen from the following results given by Löhnis as millions per gram of cheese.

	Emmental cheese		Cheddar cheese	Sour milk cheese	
	Surface	Inner part	Inner part	Surface	Inner part
Fresh curd		8	10		
After 1 day		450	30	70	70
After 6 days				300	23
After 10 days	8,500	62	41		
After 13 days				100	82
After 45 days	66,000	55	10		
After 150 days	23,000	34	0.5		

When cured at a low temperature, greater time is required for the maximum count to be reached, and the reduction in numbers comes more slowly.

Even higher results than the above are reported by Hastings and co-workers, in the following table which gives the number of bacteria in a gram of cheese:

Time of plating	Cheese number			
	580	581	582	583
Milk	8,000,000	500,000	700,000	500,000
Curd at salting	160,000,000	326,000,000	912,000,000	839,000,000
12 hours	332,000,000	1,048,000,000	623,000,000	965,000,000
1 day	586,000,000	736,000,000	709,000,000	569,000,000
2 days	235,000,000	405,000,000	848,000,000	580,000,000
4 days	145,000,000	684,000,000	522,000,000	1,025,000,000
6 days	165,000,000	184,000,000	853,000,000	184,000,000
14 days	51,000,000	211,000,000	369,000,000	401,000,000
21 days	284,000,000	290,000,000	348,000,000	319,000,000
28 days	285,000,000	453,000,000	314,000,000	144,000,000
35 days	104,000,000	261,000,000	326,000,000	504,000,000
49 days	132,000,000	288,000,000	436,000,000	661,000,000
70 days	128,000,000	291,000,000	193,000,000	168,000,000
98 days	114,000,000	212,000,000	45,000,000	55,000,000

It is quite evident that the rapid multiplication of the bacteria at first soon obliterates any differences in numbers as shown by the milk; however, the nature of the finished product is dependent upon the kind and not the number of bacteria in the milk.

A good idea of the microflora of the American cheddar cheese may be obtained from recent work at the New York Agricultural Experiment Station. Forty different samples of this cheese, all of which were obtained from different factories and from the open markets were studied, and no less than 265 cultures were isolated from them. These cultures, were subjected to the usual laboratory tests and classified into the following groups:

1. *Spore Formers.*—54 cultures. From the better grades of cheese only 9 per cent were spore-bearers.

2. *Gram-negative Rods.*—52 cultures. They were found in cheese of the poorer quality with gas and undesirable flavors.

3. *Lactobacilli.*—In the better cheese this group predominated and probably played the important rôle in its ripening.

4. *Streptococcus Lactis.*—In the early stages these organisms predominate and change the sugar to lactic acid.

5. *Cocci.*—27 strains of cocci—20 white, 6 yellow, 1 orange.

6. *Streptococci Other Than S. lactis.*—26 cultures, mainly from the poorer grades.

7. *Yeast.*—Three strains of yeast were obtained from the cheese and in one poor grade cheese the yeasts were the predominating organisms.

In the typical American cheddar cheese there is a rotation of germs during the ripening process. In the early stages the *S. lactis* type develops rapidly and may for a period be the predominating organism. In the better quality of cheese the *lactobacilli* and *cocci* develop up to the fourth or fifth month, after which the number of organisms diminish. In the poorer grades of cheddar cheese the spore-formers and gram-negative rods develop after the first initial predominance of *S. lactis.*

Change in Ripening of Cheese.—Microörganisms and enzymes are absolutely essential for the obtaining of desirable changes in the ripened cheese, for these organisms split carbohydrates, fats, and proteins and produce compounds which impart to cheese its specific texture, flavor, and aroma. During the early period of ripening, the milk sugar is transformed into lactic acid. This is indicated by an increase in the acid content of the cheese. The acid reacts with the casein and calcium compounds forming salts of lactic acid; which in turn are further acted upon by microörganisms with the production of carbon dioxide, simpler acids, and small quantities of alcohol. Some of the casein, which is the principal protein of cheese, is broken down

by the proteolytic ferments and soluble nitrogen-containing substances such as proteoses, peptones, amino-acids, and ammonia are formed. The nature and quantity of the final products vary with the kind of cheese, the time of ripening, and the temperature at which it has been stored. The splitting of the fats varies even greater than the changes in the other constituents. Fats are split principally by aërobic germs; hence, this process proceeds most rapidly in soft cheese. If carried too far it may give rise to a rancid cheese. To retard the action of aërobes, cheese is usually covered with paraffin or other substances. This is especially effective in cutting down mold growth.

FIG. 77.—Influences of bacteria on cheese. The lower row of cheeses were made from milk lacking in the bacteria essential for the proper ripening of Swiss cheese. The cheeses of the upper row were made from the same milk as their respective "blind" controls, with the addition of a pure culture of the eye and flavor producing organism. (*After Sherman.*)

Abnormal Changes in Cheese.—Many abnormal changes may occur in cheese that has been improperly made or produced from low-grade milk. Excessive production of lactic acid gives rise to a sour cheese; the excessive splitting of the fats to a rancid cheese. When the casein is greatly broken down the result is a putrid cheese. Such cheese may not only be repulsive but, are, dangerous because of the growth of putrefiers. "The fact that cheese is ripened by bacteria and that bacterial activity in the product is essential to the characteristic odor and flavor of the properly finished product does not remove the stigma of rot or decay from a product in which undesirable bacterial activity has produced odor, taste, or texture which is abnormal. Many

products of this type are constantly observed and are properly designated as rotten or decayed and should be no more permitted on the market than rotten tomatoes or rotten fish." Such products may contain the *botulism* toxin in addition to many organisms which cause illness when eaten. Jordan found that nearly 4 per cent of the cases of food poisoning occurring in the United States during 1914–1915 were due to cheese.

Foreign organisms may find their way into the cheese and give rise to inferior products. Members of the *Bact. coli* and aërogenes groups produce gas. It is the gas that cause the many

FIG. 78.—Illustrating the influence of gas producers in milk. (*After Hammer.*)

small holes in the cheese. At times the cheese may become so rounded in the center, due to the gas, that it rolls from the shelf.

Abnormal pigmentation which sometimes occurs in cheese is due to microörganisms. This coloring of cheese may take the form of red, brown, black, yellow, green, or blue spots within or on the cheese.

The use of clean milk with a uniform microflora, if it can be obtained, would prevent abnormal changes in cheese; but where milk is obtained from many dairies greatly varying in the sanitation practiced, this is impossible. The ideal method would be to use a pasteurized milk. With some varieties of cheese this

can be done successfully, but with others it cannot. The heat used in pasteurization may have an undesirable effect on the curd. Furthermore, our knowledge of the specific microörganisms concerned in the ripening of the cheese is not complete enough in all cases to warrant the use of pure cultures. The starters which have been used with varying degrees of success are sour milk, buttermilk, whey, moldy bread, and the infusions of ripened cheese. Many of these are little better than the seeding of the original milk. For cheddar cheese pure cultures of various types of lactic acid, streptococci, as well as of lactobacilli are used. The lactobacilli give a more highly flavored product than do the streptococci.

To overcome the variation which must of necessity occur from chance seeding, such as that mentioned above, and also to prevent infection and cheese poisoning, some advocate the pasteurization of the ripened product. This, when done at the proper temperature and under carefully controlled conditions, apparently gives a good product, uniform in texture and flavor, free from pathogens, and with good keeping properties.

ICE CREAM

The production of ice cream is a modern art. It has developed more rapidly in the United States than in other countries. In this country alone over 350,000,000 pounds of milk are used annually in the production of ice creams. This is about twice the milk production in Japan. As defined by the United States Department of Agriculture: "Ice cream is a frozen product made from cream and sugar with or without a natural flavoring and containing not less than 14 per cent of milk fat. Fruit and nut ice cream must contain not less than 12 per cent of milk fat. When made from clean pasteurized products under sanitary conditions, it is a valuable and highly nutritious food. If made from unclean or infected milk, it becomes dangerous."

The enormous number of bacteria which may occur in ice cream may be seen from the work of Ayres and Johnson who in 1915 collected 91 winter samples and 94 summer samples of ice cream from retail stores in Washington. None of these samples contained less than 500,000 bacteria per cubic centimeter. The average for the 185 samples was 37,000,000 the maximum 510,000,000 and the minimum 120,000 per cubic centimeter. Later Hammer made a comprehensive study of ice cream, including an

investigation of the source of bacteria, the possibilities of securing a low bacterial count, and the changes in the bacterial flora that take place during storage at low temperatures. He concluded that unpasteurized cream is the greatest source of bacteria, and may be responsible for the large bacterial count, and that sugar and vanilla extract are unimportant as a source of contamination. His investigation also showed the importance of the utensils as a source of bacteria. By sterilizing all utensils in an autoclave and by pasteurizing the cream at 60° C. for twenty minutes he was able to produce ice cream in 15 to 20 gallon-lots with a bacterial content of 4,200 per cubic centimeter. Bacteria can and do survive for long periods in ice cream. Even pathogens survive much longer in ice cream than in milk and other food products kept at room temperature. The longevity of *Bact. typhosum* in ice cream is seen from the following results reported by Purcha and Brannon:

Sample taken	Typhoid bacteria per c.c. ice cream
Before freezing	25,000,000
Freshly frozen	51,000,000
5 days old	10,000,000
12 days old	7,000,000
20 days old	2,000,000
70 days old	660,000
104 days old	900,000
134 days old	210,000
165 days old	640,000
170 days old	711,000
200 days old	60,000
260 days old	57,000
290 days old	53,000
342 days old	51,000
430 days old	30,000
544 days old	13,000
648 days old	11,000
2 year old	6,300
2 years, four months	Living typhoid germs present

Epidemics of typhoid fever have actually been traced to infected ice cream. Cumming reported 23 cases which developed among twenty-nine persons who partook of ice cream at a school picnic at Helm, California, in 1916. Ice cream was the only

food partaken of by all, and as the chocolate ice cream was the favorite flavor this was determined to be the source of the infection. This was because: (1) those not partaking of it did not become ill, (2) those partaking of it but no other food became ill, (3) those eating chocolate ice cream were taken with acute intestinal symptoms, and (4) those eating the largest quantity of chocolate ice cream were the most seriously ill.

Dysentery is also often spread by means of ice cream. Smillie studied 75 cases and found the etiology of them to be as follows:

	Cases
Contact with an acute case	21
Contact with a carrier	2
Contact with house cases	4
Condensed milk epidemic	15
Ice cream cones	9
Flies	6
Milk	1
Water	1
Fruit	1
Unknown	15

The dysentery bacillus of Flexner was actually isolated from the ice cream cones.

Hamilton has pointed out that ice cream epidemics can be prevented by: (1) the use of ingredients with a clean sanitary history, (2) the use of properly cleaned utensils and a clean factory; and (3) the proper handling of materials by individuals with a clean bill of health. The first of these is to be controlled by the pasteurization of the milk and cream; the second, by frequent inspection; of the plant and the last requires regular and careful inspection of all workers for communicable diseases.

CHAPTER XXIV

BACTERIA IN OTHER FOODS

All foods except those recently cooked or sterilized and then sealed contain microörganisms. Most of these microörganisms are saprophytes which cause food spoilage. Occasionally the food contains pathogens that may give rise to diseases in the consumer. The number and variety of germs contained in a food varies with its kind and history. Animal food contains many microörganisms, some of which may be pathogens, and may even multiply in such a food. Plant foods usually contain fewer microörganisms, and are less likely to be disease producers. The pathogens seldom if ever multiply in vegetables and fruits; consequently, the danger from these foods is not as great as in the case of animal products.

Animal Tissues.—Veal, beef, pork, lamb, fish, and fowl are among the commonest animal products used as human food. The glandular tissues of the various animals are more complete foods than are the muscle tissues; yet, the former are more likely to convey disease than the latter, and should be inspected even more carefully and cooked more thoroughly than the muscle meats. However, this should not be taken to imply that raw or diseased muscle meat should ever be used as food.

The susceptibility of lower animals to diseases which are common to man varies with the species; hence, the likelihood of these animals conveying infection also varies. For example, goats horses, dogs, and cats are quite resistant to tuberculosis and chickens are probably immune to anthrax; consequently, they do not convey them to man. Chopped meats invariably contain many bacteria, for the reason that meat used for that purpose is often that which has been discarded for other purposes. The hashing of it carries the bacteria through the entire mass which is an excellent medium for their multiplication. This is recognized by the fact that Weinzierl and Newton proposed a bacterial standard of not over 10,000,000 per gram for hamburger and then found that 50 per cent of the samples

examined had to be condemned. Hence, this form of meat is prone to produce illness.

Bacteria in Meats.—The tissues of healthy living animals are free from bacteria, but after death bacteria rapidly invade and at optimum temperature quickly decompose animal tissue. During the dressing and handling of meat bacteria are constantly being added. Some early western pioneers recognized this and killed and handled their beef and venison with great care. It was then hung in a cellar free from flies and dust and kept for weeks even in the summer months without spoiling.

Attempts have been made to set bacterial standards for the judging of meat. One authority suggested 1,000,000 bacteria per gram for the standard, and meat containing greater numbers he considered should be condemned. Others have tried to conform to this standard, but Carey showed that such a standard would condemn much of the ground market meat. At times he found that sausage often contains as many as 10,000,000 bacteria per gram. Most of the organisms occurring in meat are the common decay bacteria, but occasionally parasites and pathogens are present. The failure to find a direct relationship between the bacterial count and food spoilage and the indefiniteness of our knowledge concerning the specific causative organisms of meat poisoning has led to the rather generally accepted conclusion that bacteriological examination alone cannot be used as a criterion for the judging of meat. Antemortem and postmortem examinations of the animal and the general condition and history of the meat must all be considered in reaching a conclusion as to whether the meat should be used for human consumption or not.

Illness Due to the Use of Meat.—The excessive use of meat may injure the kidneys and possibly other organs of the body; furthermore, meat may carry bacteria or their products which may cause illness, or even death. The conditions under which meats may cause illness can be conveniently grouped under three heads:

(1) Illness may be caused by eating of meat from diseased animals.

(2) Illness may be caused by eating putrid meats.

(3) Illness may be caused by specific toxins produced in the meat, botulism.

It has been known for a long time that the flesh of animals dead from certain diseases or slaughtered while suffering from a disease is not a safe food for man. The ancient Egyptians

understood this, as is indicated by the Mosaic Law: "Ye shall not eat of anything which dieth of itself." Yet emergency slaughtering of ill or injured animals and the use of their tissue for food is not an uncommon procedure in some districts. Such meat is likely to cause illness in the consumer.

The more common bacterial infections which may be conveyed by meat to man are: tuberculosis, anthrax, symptomatic anthrax, paratyphoid, puerperal fever, pleuro-pneumonia, glanders, mucous diarrhea, actinomyces, and occasionally the illness caused by *Bact. abortus* so prevalent among cattle. The relationship between most of the above named animal diseases and human infection has been fully established, and the evidence is conclusive that in districts where uninspected meat is used they play an important rôle in causing human illness.

Gärtner in 1888 studied an outbreak of acute gastro-intestinal disease which occurred among a group of individuals who had eaten meat of a diseased cow. He isolated from the infected meat and also from the spleen of a fatal case *B. enteritidis*. Since that day, this and related organisms have been repeatedly obtained in cases of food poisoning. The organisms all belong to either the *B. enteritidis* (Gärtner group) or to the *B. paratyphoid* group. It is now generally believed that they are the cause of much of the meat-poisoning, and reach the meat by the following avenues:

(1) The animal from which the meat was obtained may have actually been suffering with the infection at the time of slaughtering.

(2) Members of both the *B. enteritidis* and *B. paratyphoid* are often found in the excretions of healthy men and animals, and healthy carriers who handle the meats often infect them.

(3) Uninfected meat may be piled with infected meat. In this way, the organisms become distributed through large quantities of meat. Moreover, these organisms under appropriate conditions of temperature multiply in meat, and a small initial infection may give rise to far-reaching effects.

It is often claimed that putrid meat causes poisoning, but the evidence for this is not conclusive. In the past many such cases were believed to have occurred and were attributed to ptomaines which were thought to be produced in the meat. Today it is doubtful if true ptomaines play an important part in meat-poisoning. Certain putrefying bacteria, such as *B. proteus*, have repeatedly been obtained from putrid meat which has caused illness, and it is held by some that they are the cause of the illness. How

they act is not definitely known at present. They may act, as suggested by Vaughan, by the production of "protein-split substances."

"The most characteristic examples of food poisoning, popularly speaking, are those in which the symptoms appear shortly after eating and in which gastro-intestinal disturbances predominate. In the typical group outbreaks of this sort all grades of severity are manifested, but as a rule recovery takes place. The great majority of such cases that have been investigated by modern bacteriological methods show the presence of bacilli belonging to the so-called paratyphoid group (*Bact. paratyphosus* or *B. enteritidis*). Especially is it true of meat-poisoning epidemics that paratyphoid bacilli are found in causal relation with them. Hübener enumerates forty-two meat-poisoning outbreaks in Germany in which bacilli of this group were shown to be implicated, and Savage gives a list of twenty-seven similar outbreaks in Great Britain. In the United States relatively few outbreaks of this character have been placed on record, but it cannot be assumed that this is due to their rarity, since no adequate investigation of food-poisoning cases is generally carried out in our American communities."

Meat Inspection.—The quality and wholesomeness of meats produced and shipped to foreign countries and across state lines for domestic consumption are probably the best meat products of the entire world. This is due to the administration by the Bureau of Animal Industry of the Federal Meat Inspection Act of 1906, supplemented by the Act of 1913. This provides for the adequate inspection of approximately 60,000,000 head of cattle, sheep, swine, and goats slaughtered annually in the United States. The inspection proceeds by logical steps, beginning with the careful antemortem examination of the animal, the inspection of the carcass while being dressed, the supervision of the curing, pickling, smoking, cooking, and canning of all meats, and finally with the proper, honest labeling of all meat products. It has been very aptly described as proceeding "from the hoof to the can," or "from the livestock pen to the finished meat or product in the labeled package ready for shipment to the consumer." The severe penalties provided in case of its violation put teeth into the act.

Approximately one-half this number, 30,000,000 head, do not move in interstate or foreign commerce; hence they do not have the benefit of federal inspection, and must be looked after by

state and city inspectors. Jones estimates that less than one-third of our cities with populations of 5,000 have any regulation of meat inspection, and that in many of these the inspection is incomplete. This is a distinct menace to the health in these communities, for the producer soon learns that he can dispose of animals on the local market which cannot meet the rigid inspection for interstate and foreign commerce.

This condition can be remedied by having the cities and municipalities pass and enforce ordinances regulating these matters. These ordinances should, in so far as they are applica-

FIG. 79.—Final inspection of dressed beef. (*Courtesy, Swift and Company.*)

ble, be modeled after the Federal Meat Inspection Law, and should provide for both postmortem and antemortem inspection of all animals to be used for human consumption and severe penalties for violation of the regulations. In small communities where possible expense can be reduced by coöperation in the passage and enforcement of the act. The city and county of San Francisco has an ordinance which may well be used as a model.

Prevention of Meat Poisoning.—Proper public meat inspection will do much to prevent meat-poisoning, but the consumer must also coöperate. It is quite evident that spoiled meat should not

be consumed, but it is not always easy to recognize putrefaction in the early stages. However, it is well to remember that the process commences at the surface and spreads to the deeper layers. There is a tendency for spoilage to first manifest itself along fibrous tissue or the bone. The cut surface of spoiling meats usually appears porous; indentation made with a blunt object persists; the fat changes in color; the bone marrow becomes soft. There is a foul odor which is most marked about the bones and in the fat. The odor becomes more pronounced during cooking and does not disappear from the fully cooked meat.

Meat should be kept in a properly iced refrigerator, and the refrigerator should be frequently and thoroughly cleansed with hot soda solution. Chicken, fish, and chopped meats are especially apt to spoil, and it is a good precaution to thoroughly cook all meat before it is eaten. This will destroy all pathogens and destroy or weaken most toxic bacterial products which may have been produced in the meat.

Bread.—The importance of bread to mankind is voiced in the supplication: "Give us this day our daily bread." Wells states "Secondary foods may be more important than bread with certain classes of society—the rich, for example—in certain localities, and at certain limited periods; but throughout civilization and beyond, bread and bread alone is the basic common food. What milk is to the infant, bread is to the world." The nature and composition of bread varies greatly in different localities and with different people, but in all cases it is the commonest food and made of the commonest cereal of the land. Its biological interest comes from the methods used in leavening, the spoilage due to microörganisms, and its likelihood of carrying human pathogens.

From start to finish bread-making is a biological process, and, as pointed out by Wells, it is the use of a leavening agent that has transformed wheat from the poorest to the best bread grain.

"Since he first found out how to use fire to cook his food, man has made but two other really important food discoveries. These are the preserving of meats and other perishables by salt, smoking, or drying, and the use of leavening in breadmaking. These two discoveries are both pre-historic. The importance of leaven in bread-making cannot be over-estimated because without leaven, wheat and rye would never have become leading food grains. Without leaven the cultivation of wheat and rye would now cease. Without leaven wheat is the most stubborn and intractable

of possible foods. Even an amateur cook can make an edible bread from Indian corn, or barley flour and water without leaven, but a professional would be stumped to make anything edible from wheat flour and water alone. But with leaven, yeast, or baking powder, wheat becomes the supreme bread-making grain. In other words, wheat, the least suitable of all grains for use as a human food, with leaven becomes the most suited. This is because it is best suited for making that kind of food which is the base food of all civilized peoples. Here we are at the root of one of the most significant facts of present day civilization. Man by the discovery of leaven has been able to raise one grain, and

FIG. 80.—Bread made from flour of different gluten content.

that in its natural state, the least promising of all grains, to be not only the prime grain but the prime food as well."

At first the leavening agent was that which chanced to adhere to the grain; later it became the custom to add to new dough a bit of old fermented paste or "leven," and still more recently yeast was substituted for the piece of leaven.

Some believe that the main function of the yeast is to aërate the bread and the famous German chemist, Liebig, at one time advocated chemical aërating agents. He laid great stress on the loss of nutrients due to the use of microörganisms. But we now know that profound changes are wrought by the microörganisms on the carbohydrates and especially the proteins of the flour, and the lightness, sweetness, and digestibility of yeast made bread surpasses by far that made with the use of chemical aërators.

Yeast.—The main leaven used in bread-making today is yeast. The following is a convenient classification of the yeasts:

I. *Saccharomyces Cerevisiæ.*—These are the yeasts generally used in the manufacturing of bread and are usually divided into three groups:

a. Bottom yeasts of German beers.
b. Top yeasts of English beers.
c. Distillers yeasts producing larger quantities of alcohol than the beer yeasts.

II. *Saccharomyces Ellipsoideus.*—These yeasts occur naturally on the grapes in the vineyard and are sometimes called wild yeasts. They are used to some extent in the making of wines and distillery products. In some countries they are used in bread making.

III. *Torula, pseudo,* or false yeast. These are the trouble makers in the fermentation industries. They produce disagreeable bitterness; unpleasant flavors, persistent cloudiness, and the like.

The leavens used in bread-making may be roughly divided into three classes.

1. The comparatively pure yeast obtained from distillers' wort and which is placed on the market as compressed yeast, dried yeast, and liquid yeast.

Compressed yeast is collected from the wort, thoroughly washed, passed through wire and silk sieves in order to free it from foreign material, and pressed. It is then cut into cakes and wrapped in tin foil. This form of yeast keeps but a short time and spoils quickly; so it must be kept cold. Dried yeast is made by mixing fresh liquid yeast with cornmeal, flour, or other starchy materials, compressed into cakes, and dried at a carefully regulated temperature. If properly made this yeast keeps for a considerable time. The percentage of living cells in dried yeast is small as compared with the compressed yeast, and the numbers gradually decrease with time. Liquid yeast is collected directly from the distillers' wort and dispensed as a concentrated liquid. It keeps poorly and is likely to be contaminated with bacteria.

2. The yeasts propagated in the bakery, or the home. These are made by growing yeast on media made of potatoes, sugar, hops, and various other constituents.

3. The spontaneous leaven which develops in mixtures of flour and water when kept under appropriate conditions. These are mixtures of yeast and bacteria which occur on the grain. Comparatively large quantities of salt are often added to prevent the growth of the high acid-producing bacteria.

Biological Changes during Leavening.—Carbon dioxide is evolved which gives porosity to the bread. Some of the starches and proteins are split and are then more easily assimilated by the body. The gluten is softened and certain characteristic flavors and aromas are developed.

Abnormal Fermentations of Bread.—The most common abnormal fermentations in bread are ropiness, undesirable high acidity, and "bloody" bread. Ropiness, or sliminess in bread results after baking from the growth in the bread of certain slime-forming highly resistant spores. These may have come from the yeast, the flour, or the various utensils used in the bread-making process, and when they get into the bakery become a real nuisance. The acid developed in normal fermentation tends to prevent their growth, as does also the keeping of the bread at not too high a temperature. Sour bread sometimes results from excessive growth of lactic and butyric acid bacteria. They may reach the dough in excessive numbers from the flour, water, and impure yeast. They give to bread bad flavors. Large quantities of good yeast tend to keep them in check. Colored spots may occur in bread due to the growth within it of chromogenic bacteria. One of the most common is *Erythrobacillus prodigiosus* which causes the so-called "bloody" bread.

Bread and Diseases.—Unwrapped bread if carelessly handled may become infected and convey human infection in this manner; but the modern automatic bakery machinery along with the automatic wrapping machine makes it possible for the baker to guarantee the public bread free from disease germs. Consequently, today many bakers truthfully advertise "bread which has never been touched by human hands." Such bread would not convey pathogens.

Confections.—"The confections are members of a large group of foods which are often handled in such a manner as to cast doubt on their healthfulness. They are often dispensed by dirty hands, from receptacles, and kept in stores of questionable cleanliness. They are usually eaten without further preparation which would render them free from any undesirable bacteria, should they be present." The poorest grades and often the least sanitary forms are consumed by children who are highly susceptible to both the poisonous ingredients and any infection which they may carry.

Tanner and Davis analyzed thirty samples of candy which they purchased on the open market. The bacterial content ranged

from 0 to 2,240,000 per cubic centimeter. They found that the bacterial content depended more upon the presence of certain constituents than on the method in which they were dispensed. Fudge, and those containing soft centers, chopped figs, and cocoanut had high bacterial contents. The real hard candies were lower, and candies containing peppermint and other essential oils were still lower, due to the germicidal properties of the flavor. Even frail microörganisms do not readily die in or on candy. Fudge was prepared in the ordinary manner and then heavily seeded with cultures of *Bact. coli* and *Erythrobacillus prodigiosus*. After four months storage under dry conditions living cells of both organisms were isolated. Cummings found that *Bact. typhosum* when inoculated in large numbers into chocolate survived for 140 days. Such experiments indicate that candy should be handled under sanitary conditions and by healthy individuals; otherwise, it is a menace to the public health, for disease carriers, if allowed to prepare or vend candy, might readily spread typhoid, tuberculosis, or other diseases.

Explosions in Chocolate Candies.—The chocolate coating on candies sometimes cracks and at times is broken into a number of fragments. This occurs in from ten to fourteen days after the candy has been manufactured, and apparently the manufacturer is unable to foretell the trouble. It occurs only in chocolates having a fondant center, which is composed of egg white, sugar, flour, flavoring, and other constituents. It has been shown that this spoilage is due to the growth of gas-producing microörganisms within the candy. These bacteria have their origin in the egg, starch, and other constituents used in the making of the candies. The organisms which have been incriminated in this connection are the various gas producers: *Bact. coli*, *B. Sporogenes*, and *Cl. multifermentans*.

CHAPTER XXV

BOTULISM

The most fatal form of food-poisoning known comes from the ingestion of toxins elaborated in the food by *Clostridium botulinum*. It was early associated with sausage poisoning from which it derived its name. However the name has lost its original significance, for it has been shown that the organism grows and produces its toxin in many other foods. It is unique in being a very resistant spore-bearing anaërobic saprophytic microörganism producing one of the most potent toxins known.

Historical.—For many years botulism, or as it was first known, sausage-poisoning, has been distinguished from other forms of food-poisoning. This has been due to its high mortality, its peculiar symptoms, and its association with the consumption of sausage.

For nearly two hundred years Würtemberg, Germany was the reputed birth-place and home of sausage-poisoning. Varied were the interpretations which were given as to the cause: some thought it to be brought about by copper which was supposed to have come from the vessel in which the sausage was prepared; while others, believed it to be caused by belladonna or henbane which had been added accidentally or intentionally. In 1892 official instructions were issued concerning the preparation of sausages. In these it was held that metallic poisons play no part in the poisoning, but the poison was carried into food by various imported condiments used to give it flavor. In the twenties of the nineteenth century, a German poet and medical writer, Kerner, enumerated 171 cases with 75 deaths which had occurred in various epidemics in Würtemberg. He considered they were due to the great consumption of large sausages which had been made by primitive methods from inferior materials; consequently, it came to be suspected that some deleterious change occurred within the sausage itself, thus rendering it poisonous. One writer attributed it to a poisonous fatty acid, another to acrolein, and still another considered that there was produced within the sausage hydrocyanic acid and that this was

the causative agent. It was even suggested by some that the meat had come from sick animals, and that the cause of the trouble lay there. There were also those who suggested that toxic substances were produced in the meat during the smoking. However, the true etiology of botulism was not established until 1894 when Van Ermengem, following an epidemic in Belgium, obtained from the suspected food and from the spleen of one of the victims, *Cl. botulinum.* Since that time it has been repeatedly demonstrated that this is the causative organism.

Botulism was long regarded as a rarity in America, but since 1912 numerous cases have been recognized, as may be seen from the following table by Geiger, which gives a record of human botulism in the United States since 1920.

Year	No. of outbreaks	No. of cases	Deaths	Mortality (per cent)
1920	14	48	33	68.7
1921	14	56	22	39.3
1922	21	55	46	83.6
1923	12	21	16	76.2
1924	8	43	29	67.4
1925	8	21	15	71.4

The outstanding characteristics of these cases are the high mortality, the rather restricted areas in which they have occurred, and the fact that most of them have been caused by home-processed foods. Probably the cases were greatly increased by the extensive use of the cold-pack method which came into favor during the war. It is just possible that many of the earlier so-called cases of "ptomaine poisoning" reported in this country were botulism.

The occurrence of recognized cases in the United States has stimulated research in this field, which has shown: (1) That botulism is endemic within regions of the United States. (2) It results from the ingestion of certain improperly processed fruits and vegetables in addition to meats. (3) Forage poisoning in horses and other animals may at times be due to botulism. (4) The causative organism is a saprophyte and may occur in native soils. (5) There are different strains of the organism which, although similar morphologically, produce specific toxins.

FIG. 81.—Clostridium botulinum. (*Courtesy, Dr. Dozier.*)

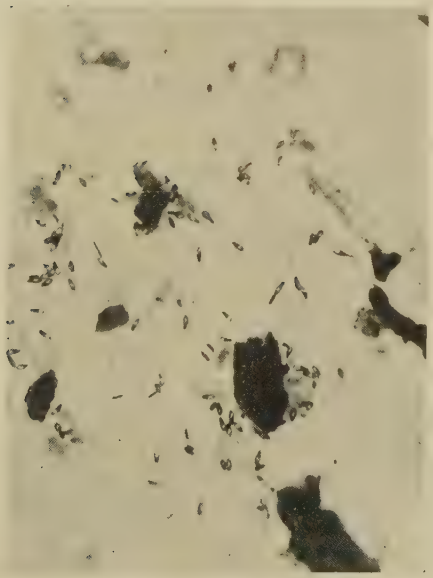


FIG. 82.—Clostridium botulinum showing spores. (*Courtesy, Dr. Dozier.*)

Causative Organism.—*Clostridium botulinum* is a large, spore-bearing anaërobe. It is usually described as being from 4 to 9 microns in length and from 0.9 to 1.2 microns broad. Generally the ends are rounded, and often two or more individuals are attached end to end, occasionally forming long threads. It is motile with 4 to 8 flagella at the pole, and forms resistant subterminal oval spores. The maximum heat resistance of numerous strains tested by Meyer in a neutral phosphate solution was found to be:

4 minutes at 120° C.
10 minutes at 115° C.
100 minutes at 110° C.
330 minutes at 100° C.

Although *Cl. botulinum* is an anaërobe and grows best when all oxygen is excluded, yet it grows well under aërobic conditions in the presence of other organisms which consume the oxygen. Thus, it may grow and elaborate its toxin in other than canned foods. The organism grows well in a great variety of media, and meat extracts are not essential for its growth and toxin production. It grows readily at temperatures ranging between 20° and 40° C., but the greatest toxin production takes place at approximately 37° C. Small quantities of sodium chloride seem to be essential for its growth, but large quantities are injurious as growth ceases in a 6 per cent salt solution, but not in 50 per cent syrup.

Distribution.—*Clostridium botulinum* is widely distributed in nature. Burke found the organisms in the following substances: (1) bird-pecked, bruised, and moldy cherries; (2) bean leaves covered with spots or droppings of insects or small animals; (3) spiders from bush bean plants as well as on the bush bean; (4) moldy hay upon which horses and mules had fed and later developed botulism. Although Burke's work was confined to California, other workers have shown that the organism is widely distributed elsewhere. It is thus possible to contract botulism from the eating of a great variety of improperly processed foods from many localities.

Meyer and his co-workers made the surprising discovery that the organism occurs most frequently and most abundantly in virgin soil. It was found in soil from mountain sides where there was no evidence of soil pollution by either man or animal. They further found that type A is more prevalent in virgin soil, whereas type B predominates in soil that has been cultivated. The dis-

tricts most heavily infected by type A as shown by the work of these investigators are the mountainous and coast states of the West, including Washington, Oregon, and California, with Utah, Wyoming, and Montana slightly less infected. Type B. seems to predominate in the East. These findings led Meyer to conclude that the original and natural habitat of *Cl. botulinum* is mountainous virgin soil, from where the organisms are transported to the lowlands by water, wind, and birds. As a result of the changed environment, there is a mutation of type A to type B. The organism has been found on fruit and vegetables in the infected districts to the extent of 30 per cent of the samples tested. It is the toxin which causes illness; hence, there is no danger in eating fresh fruits and vegetables even when they carry spores of *Cl. botulinum*, since they are not accompanied by the toxin.

It was believed that the organism grows and produces its toxin only in meat, until Dickson showed that fruits and vegetables are also suitable pabulum. Since then a number of outbreaks have occurred, resulting from the consumption of improperly processed beans and olives. The organism apparently grows well and elaborates a strong toxin in silos, for it has occasionally been the cause of forage poisoning.

Pathogenicity.—It is generally stated that *Cl. botulinum* does not multiply and elaborate its toxin in the bodies of animals, and clinical observations confirm this view. However, recent laboratory experiments have shown it possible to produce fatal botulism by feeding massive doses of toxin-free spores, thus raising anew the question: May spores germinate within the body and form a virulent toxin? If they do, there is a possible danger in the eating of food which contains many spores of *Cl. botulinum* even though the toxin has been destroyed by cooking.

Botulism, due to the ingested poison, may occur not only in human beings, but also in horses, mules, and cattle. Chickens often die in large numbers after feeding upon discarded food containing the toxin. The peculiar symptoms which develop have caused the malady to be referred to as "limberneck."

Botulism in cats, dogs, hogs, and goats is rare, though cases have been reported. Rabbits, guinea pigs, mice, and apes are susceptible to experimental injections or feeding of the toxin.

Symptoms.—The symptoms develop only after an incubation period of from 12 to 24 hours, the time varying with the quantity of toxin ingested. The most constant and characteristic

symptoms are: (1) The mouth and throat become dry; the skin is usually dry at first, but later is rough and harsh. (2) The motile activity of every part of the alimentary tract is lessened; there is usually constipation and retention of urine without vomiting. (3) There is a general enfeeblement of all voluntary muscles. (4) There is an absence of fever and little interference in the early stages with the higher nervous centers; hence, no delirium. (5) In many instances there are scintillations before the eyes, dimness of sight, and double vision. Often this is the first indication the patient has that anything is wrong. (6) There is a dropping of the eyelids, and there may be respiratory or circulatory disturbances which threaten or actually end life. Loss of voice and difficulty in swallowing is often manifest. (7) The pulse is usually slow, but recent cases in the United States report a rapid pulse, in some instances as high as 150 per minute.

The duration of the disease varies widely. Cases have been reported which have lasted as long as one hundred days. In fatal cases death usually occurs about the tenth day. The mortality of botulism is high, in the United States, as stated by Dickson, it has been over 64 per cent.

Toxin.—The toxin is produced under strictly anaërobic conditions. However, even when grown in mixed cultures, the organism may produce its toxin because of the fact that the associated organisms withdraw the oxygen from the vicinity of the anaërobe. Although it will grow and produce toxin in a slightly acid medium yet, according to Dickson, it is much more potent if grown on an alkaline medium and in the dark. In the early history of the subject, it was believed that animal protein was necessary for the production of toxin. Later work has shown that this is not necessary as the toxin is readily produced in string beans, peas, other vegetables, and even some fruits. The best temperature for its production is between 28° and 37.5° C. It is destroyed when exposed to a temperature of 80° C. and also by sunlight and air, but will maintain its potency for six months if kept in the dark away from oxygen as would be the case in preserved food.

Botulism toxin is one of the most potent poisons known. Von Ermengem reports an outbreak where 200 grams of poisonous beans caused the death of a human being. He cites another case in which a piece of preserved duck the size of a walnut caused the disease to last six weeks. In other cases given by him a patient died after tasting a small spoonful of spoiled corn; another died after "nibbling" a portion of a pod of spoiled string beans. Still

another was made severely ill by simply tasting but not swallowing a pod of spoiled beans, all of which indicates that it is not safe to even taste meat or vegetables which are suspected of being spoiled.

The toxin has never been obtained in pure form, but investigators have prepared it so potent that 0.000,001 of a cubic centimeter would kill a 250-gram guinea pig in four days. It is so effective that only a few molecules are required to kill small experimental animals. The toxin may be absorbed into the body from the mouth or the intestines, but probably the greater portion enters from the stomach.

Antitoxin.—The toxin of *Cl. botulinum* is similar to that produced by the tetanus and diphtheria bacilli in that it is a true exotoxin, and if injected into animals in small increasing doses immunity results. This has been made use of in preparing an antitoxin which is quite effective in protecting experimental animals against botulism. Where it has been used on human individuals the results have been encouraging, but the number of cases in which it has been used is small. The antitoxin is effective only when administered previous to the onset of symptoms; hence, it does not neutralize the poison after it has once combined with nervous tissue. It appears to have some effect when taken through the stomach but is most effective when given directly into the blood stream. The antitoxin produced by one type protects only against itself; consequently, the type must be determined, or the antitoxin for all three types given.

Prevention.—The prevention of botulism at the present time rests upon the following principles; (1) Only sound unspoiled fruits and vegetables should be used for canning. These should be handled in a sanitary manner and processed with reliable methods. The cold-pack method is not reliable. Complete sterilization results only with the pressure method, and then the time and temperature must be such that the entire content of the can is sterilized. The latter naturally vary with the size of the container and the nature of the products. Where products are preserved by means of salt, not less than 10 per cent of salt should be used. (2) The housewife should learn the signs of spoilage and carefully examine every jar before using its contents. The signs of spoilage are: (a) The blown lid, the squirting of liquid when the cap is removed and the presence of gas bubbles in the jar. (b) The odor of butyric acid, (rancid cheese) the odor becoming more pronounced on standing. (c) The mushy appear-

ance of the solid parts of the food in the jar. All cans presenting any or all of the evidence mentioned above should be destroyed. It is not safe to taste the product to find out if it is spoiled. (3) All food processed by unreliable methods and those suspected of spoilage but showing no sign should be boiled for 30 minutes before using or even before tasting. (4) Salads should not be prepared and then kept for long periods at room temperature before using. The toxin is not produced when kept at low temperatures. The careful systematic application of these principles will greatly reduce if not entirely eliminate all cases of botulism.

CHAPTER XXVI

FOOD PRESERVATION

Modern thickly populated cities have been made possible through the development of rapid means of transportation and the extensive preservation by physical and chemical means of perishable foods. Today food is quickly transported hundreds or even thousands of miles from the producer to the consumer. The bounteous harvests of one season are preserved for the season of scarcity. The tables of civilized man today bear a greater variety of food than ever before. Modern developments have made possible the serving of Australian meats in London, California fruits in New York, Wisconsin milk in Manila, and eggs from China on the Chicago market. Today man receives a greater variety in his diet than at any previous period, and were it not for the ever-growing tendency to use highly refined food products this would guarantee to him a more nearly balanced diet. However, the greater distance between producer and consumer, which increases the opportunities for infection, and the more general use of food preservatives also multiply the sanitary problems which confront the health workers.

Methods of Preserving Food.—Food may be preserved by heat, cold, drying, concentration, and chemicals. The various methods and food usually preserved by each method are given in tabular form below:

I. Preservation by Temperature Changes:
   1. Heat:
      (*a*) Boiling (Most fruits and some vegetables in glass containers)
      (*b*) Steam Pressure (Vegetables, milk, etc., in tin cans)
      (*c*) Pasteurization (Market milk, beer, wine, fruit juices)
   2. Cold:
      (*a*) Freezing (Meats, milk, ice cream, etc.)
      (*b*) Refrigeration (Low temperature but not frozen) (Vegetables, fruits, beverages, meats, dairy products, etc.)

II. Drying:
   1. In natural state (Raisins, currants, and other fruits)
   2. In powdered form (Eggs, gelatin, milk, etc.)
   3. After curing (Various meats)

III. Concentration:
    1. Sugars (Jellies, syrups, jams, preserves, milks)
    2. Salt (Fish, meat, vegetables)
    3. Pickling (Pickles, sauerkraut, beans, etc.)
IV. Chemicals:
    1. Spices and essential oils (Fruit cake, mince meat, condiments, etc.)
    2. Other organic chemicals referred to as food preservatives (Many foods may be preserved by the use of benzoate of soda, sulfurous acid, etc.)
V. Excluding of oxygen:
    1. Eggs, fruits, etc.
VI. Filtration:
    1. Liquids, including media of various kinds.

Heat.—The preservation of food by heat is nearly as old as the use of fire for the cooking of food. However, in the early days it was used only occasionally and then in a haphazard way. It was given a great impetus by the work of Pasteur. During the middle of the nineteenth century the French wine industry, which had an annual value of five hundred million francs, was threatened by what was known as the "disease of wine." The wine industry faced a crisis, and it appeared that French wines were about to lose their world-wide reputation for flavor and bouquet. Pasteur moved his laboratory into the heart of the afflicted district and after much experimentation found that heating the wine to a temperature of 55° to 60° C. for a few minutes prevented it from spoiling without harming the delicate flavor of the product. This method was tested on a considerable scale by order of the Naval authorities. The ship, Jean Bart, before starting on a voyage took on board two hundred liters of wine, half of which had been heated under Pasteur's direction. At the end of ten months the heated wine was mellow and had a good color, whereas the other had an astringent almost bitter taste. Other more extensive tests with wine gave the same results. In this manner began the modern method of pasteurization which saved for France not only much money but has since also saved untold thousands of human lives throughout the world as a result of the commercial pasteurization of milk.

Pasteurization is carried out in the modern milk plant by heating the milk to a temperature of 65° C. and holding it at that point for 30 minutes. Such a procedure guarantees to the consumer a milk free from pathogens. In modified forms it is also used in treating cream, wine, beer, fruit juices, and vinegar.

Heating to the boiling point is a method quite generally used in the home for the preservation of fruits and to a lesser extent of

vegetables. This may take the form of the cold-pack or open-kettle method, the efficiency depending to a great extent upon the nature of the product and the skill of the operator. Acid containing foods are more effectively handled by these methods than are the neutral products. Usually the finished product is not sterile, but the anaërobic conditions under which it is kept prevents the germination of all molds and many of the bacteria.

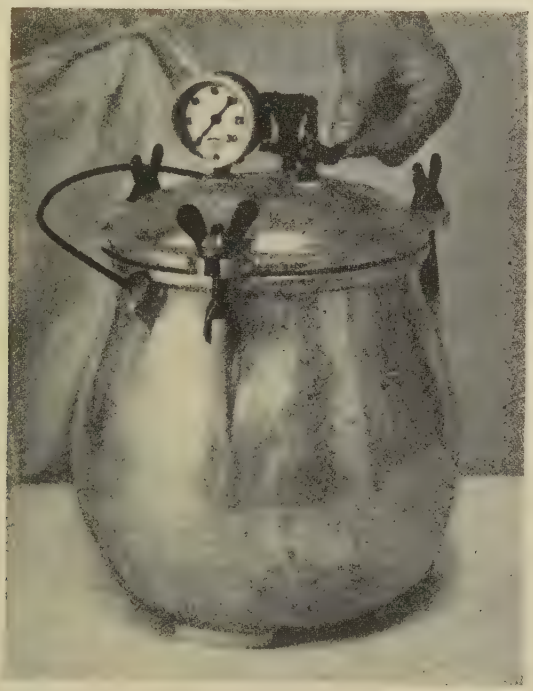


FIG. 83.—Aluminum pressure cooker, very acceptable for home-canning.

Often the intermittent method of sterilization is used to increase effectiveness. This consists of heating the containers with their contents on three successive days, thereby, permitting the germination of spores which escaped the first or even second heating. But even this does not insure a sterile product as the time and conditions are not favorable for all the microörganisms to germinate which may be in the product; hence, commercial canning is usually done by means of steam under pressure. Food-

poisoning which has occurred during recent years has made some workers advocate this method even for home-canning.

The temperature necessary for an effective "process" varies with a number of factors, chief of which are: (1) The nature of the food. Acid products are more easily processed than are neutral products. (2) The size of the can and the nature of the fill. (3) The rate of heat penetration for the particular product.

Even commercially canned foods are not always sterile, for it has been repeatedly shown that even cans which show no signs of spoilage contain living microörganisms. Most of these are in the spore state and are restrained from growth by the anaërobic conditions. The aim of the manufacturer is to place on the market a product which will keep and also appear as attractive as possible. The growing demands for an attractive product has led in some cases to the use of glass containers. This may be accompanied by some danger, as recent work has shown that much of the cheap glass containers on the market will not stand the high temperature of processing without chipping. Bits of glass may, thus, find their way into the food products. The principles underlying successful home-canning and the use of canned products is summarized by Thom as follows:

1. Only clean, fresh, sound products should be used for canning. It is hard to sterilize dirty or partly spoiled products such as stale, fermenting, souring, heating, slimy, wormy, specked, or partly rotted fruit or vegetables.

2. Keep a record of every can, its contents, and methods of handling.

3. Hold all cans under observation in the kitchen or pantry until you are sure that they are keeping. This means for a period of not less than ten days.

4. Some cans will spoil on account of faulty containers, accidents, under-cooking, or mistakes. Before even tasting, inspect every can; destroy the bad, recook cans of that lot or cans treated in the same way and apparently good.

5. Inspect at opening time:

In Tin

1. Both ends should be flat or curved slightly inward. Neither end should bulge, snap back when pressed, nor feel loose. *Make no exceptions.*

2. All seams should be tight and clean, with no trace of leaks.

In Glass

1. The cover should be firm—flat or concave with seam, rubber rings, and label clean and free from all signs of leaks.

2. The contents should appear free from molds, disintegration, cloudiness, or other abnormality, and show no discoloration when opened. Suction inward is highly desirable. No outrush of gas or spirt of liquid should occur.

The odor, observed immediately, should be characteristic of the product. No trace of a foreign or objectionable odor should be present. No disintegration, no mold, or other abnormal appearance should be observed. Liquid enough to cover the food is desirable in most products.

The inside of a tin can should be clean and bright or well lacquered, not extensively blackened or markedly corroded. If you know it is spoiled, destroy it. If after examining it, you cannot tell, add half the volume of boiling water and boil thoroughly. Canned vegetables should be boiled as an added precaution.

Cold Storage.—An ideal method for preserving food should retard natural metabolism in the food product to such an extent that it does not become over-ripe during the storage period. Furthermore, it should prevent bacterial changes without impairing the appearance, flavor, or nutritive value of the product. These conditions are more nearly met by refrigeration than by other means of food preservation. Cold, as a rule, does not destroy bacteria, for they will survive very low temperatures, but foods kept at low temperatures for considerable periods may show a decrease in the harmful bacteria. Although refrigeration does kill animal parasites, it is often not sufficient to even prevent multiplication of microörganisms, for Brooks in studying meat in cold storage isolated several organisms capable of growing at temperatures of −6° C. Other organisms have been found to grow at temperatures even lower than this provided the medium in which they are held does not crystallize. Low temperature has a selective action and prevents the growth of pathogens. Rosenau found that the organisms growing in milk at low temperatures are mainly putrefiers, and doubts that they produce toxic products. Refrigeration retards, but does not completely stop bacterial changes. The slowing down of bacterial multiplication is seen from the following results given by Russell. The time given is that required for a generation of *Bact. coli*.

45° C. 20 minutes	25° C. 40 minutes
40° C. 17.2 minutes	20° C. 90 minutes
35° C. 22 minutes	15° C. 120 minutes
30° C. 29 minutes	10° C. 14 hours 20 minutes

From this we see that cold not only reduces the number of microörganisms produced, but it does very materially retard the speed with which they acted upon the product. The psychrophilic organisms are able to function at much lower temperatures

than *Bact. coli*, and it is quite probable that it is these microörganisms which bring about the major changes occurring in cold storage foods.

It is evident that the effectiveness of food preservation by this method depends upon the temperature maintained. The home refrigerator, which is often placed for convenience in the kitchen near the range, may have a temperature near optimum for incubation of some microörganisms. Where ice is used for refrigeration, the temperature seldom reaches zero. However, with the modern mechanical means much lower temperatures are maintained. The temperature to be used depends to a great extent on the products to be kept. Some foods such as ice cream and fish must be kept at temperatures below zero to maintain the texture and to prevent spoilage. Eggs, fruit, and similar products must be kept at a higher temperature, for low temperatures cause deterioration of the products.

The temperature at which fruit and vegetables are stored varies with the product, but as a general rule it may be stated that the best temperature at which to hold the storage room is just above the temperature at which the fruit freezes. Some vegetables, for example squash, may keep better at a higher temperature. In all cases ventilation and humidity must be carefully controlled if the best results are to be obtained. Fruit is often wrapped to prevent the spread of fungi and wilting.

Ice cream is usually stored in the hardening room which has a temperature of about -9° C., whereas, meat may be stored slightly above or just below freezing temperature. The higher temperature permits the ripening processes to go on and does not interfere with the normal texture and appearance of the meat. Meat treated in this manner should be stored in a dry atmosphere so that moisture will not collect on its surfaces and permit the multiplication of bacteria. When frozen, the texture of meat, as well as its appearance and flavor are changed. Frozen meat on thawing rapidly spoils due to the oozing out of the serum, the looser texture, and to the rapid multiplication of the organisms which have slowly penetrated and seeded the frozen meat.

Fish is successfully stored for considerable periods only when frozen. Still better results are obtained by dipping it into water and refreezing so that the surface is covered with a layer of ice. This prevents the growth of psychrophilic bacteria at the surface.

According to Pennington from 75 to 90 per cent of all the poultry produced in the United States is placed in cold storage, most

of it for only a few weeks or months, but occasionally it is stored for years. The United States Department of Chemistry has shown that under such long storage, the tissues become thickly seeded with bacteria and undesirable changes occur. These are described by Pennington as follows:

"In the cold storage of chickens it is generally conceded that the fowl should be frozen as soon as possible. Some warehouses place them for a few hours at a temperature of −23° C. and then transfer to a temperature of −9.5° C. for permanent storage.

FIG. 84.—Beef hung in chilling room. (*Courtesy, Swift and Company.*)

Special care is used in the thawing when brought out of cold storage.

"The most striking difference between this chicken stored for three years and those stored for shorter periods, or those which are fresh is a pronounced inflexibility and the general green tint of the skin. The whole appearance of the bird was unpleasant in the extreme. The odor was not that of putrefaction, but was of a sharp, penetrating, unpleasant character, having a biting property, which suggested the effect of acrolein on the eyes and nostrils." The skin, muscles, and internal organs all showed profound decomposition changes.

Drying.—Preservation of food by drying was one of the earliest methods used, and within recent years has been greatly extended and improved. This is exemplified in terms used to refer to the various preserved food products. The term "dried fruit" is applied to any product in which the moisture is reduced by exposure of the fresh material to the heat of the sun, whereas when the moisture is removed by artificial means the product is known as an evaporated or less often as a dehydrated or dessicated fruit. The term "dehydrate" is used to imply the removal of water without materially altering the constituents within the food product. By this means many fruits, vegetables, meats, and even the highly perishable products, milk and eggs, may be preserved.

Drying when compared with refrigeration is seen to have many distinct advantages. (1) It yields, as Banks points out, a convenient product. "The possibilities in drying food products are enormous. One case may be cited. In preparing a vegetable soup or stew, the cook may choose from a dozen vegetables, picking out whatever combination or quantity is needed for the particular dish wanted. Powdered onion, garlic, mint, peppers, and other flavors are quickly available by the same method. Any quantity may be used whenever it is wanted." (2) The removal of great quantities of water greatly facilitates storage and shipment. For this reason dehydration of foods was greatly accelerated by the World War. It is especially suitable for products such as milk which may have to be shipped great distances. (3) All bacteria require moisture for multiplication, and if desiccated soon die. The time they can survive varies with the medium, extent of desiccation, and the specific microörganism. Most pathogens are quite susceptible to drying. (4) Food products sufficiently dried and sealed may be kept for indefinite periods under great climatic variations without deterioration. (5) The removal of the water may at times render certain constituents insoluble. Hence, it is not restored to its original condition by the addition of water. However, attempts are being made to avoid this in the modern means of dehydration. (6) Some products on dehydration lose in appearance and flavor, many fruits become dark. These are often bleached by sulfur fumes. The sulfur also acts as a disinfectant and assists in the prevention of the growth of microörganism. It may, however, be objectionable in that it renders the food less digestible. (7) Drying is without effect on the nutritive value of most foods. Vitamins A and B are not affected, but the oxidation process

going on in the dried product may reduce the efficiency of vitamin C.

Fruits.—The ease with which fruits are preserved by drying varies widely with the variety, depending upon the concentration of the soluble constituent held within their juices. Grapes and prunes keep well when containing considerable water. Apples, peaches, and apricots keep less easily, whereas some berries and other fruits are dried with difficulty. Raisins and currants are best dried by the heat of the sun, whereas some products can be successfully dehydrated only by artificial means. The great objection to natural drying is the uncertainty of the weather, dust, and insects. With modern machinery it is possible to peel, core, and slice apples at one operation. They are then dipped for a few minutes in a weak solution of salt water to prevent the discoloration which would otherwise occur when the flesh of the apple is exposed to the air. "After dipping, the apples are commonly placed in the drying trays, in which they are later taken to the drying machine. Many manufacturers subject them at this stage to a short fumigation with sulfur the purpose of which is to make the color lighter and to kill any moth eggs or injurious microörganisms which may be present in the fruit. Sulfuring, which is used with various kinds of fruits, is in this country carefully regulated so as not to harm or harden the fruit, which when dried should be soft and pliable; on being removed from the dessicator the fruit is allowed to stand, for what is known as the "sweating," to take place, a process which usually requires several days and is carried out in the open air or well-ventilated chambers." Only fruit free from spoilage should be used for drying and the finished product should be handled in a sanitary manner.

Meats.—Meats may be dried either directly or after some preliminary treatment. In the hot districts of South America and the Western Plains of the United States, beef is often cut into strips and then exposed to the heat of the sun until sufficient water is removed so that it will keep. At other times it is dehydrated by artificial means and placed on the market as a powder. Either product keeps well and is highly nutritious.

Pickling.—Cabbage is often preserved by slicing into large vats and covering with layers of salt. The salt serves a two-fold purpose: (1) Due to the osmotic pressure it extracts from the cell the sap, which offers a very suitable medium for the growth of the lactic acid bacteria. (2) Competitive microörganisms are

checked, whereas the lactic acid organisms are not influenced by the concentration of salt used (about 2 per cent). Hence, there results a microflora composed of lactic acid bacteria and yeasts. These break down the tissues of the cabbage with the production of various acids. Properly prepared sauerkraut is light in color with a brittle texture; occasionally it develops a pink color due to the development within it of pink bacteria. This condition seems to be favored by excessive quantities of salt.

Preservation by the Use of Salt.—Beef, pork, fish, and many vegetables such as cucumbers, cabbage, cauliflowers, beans, and

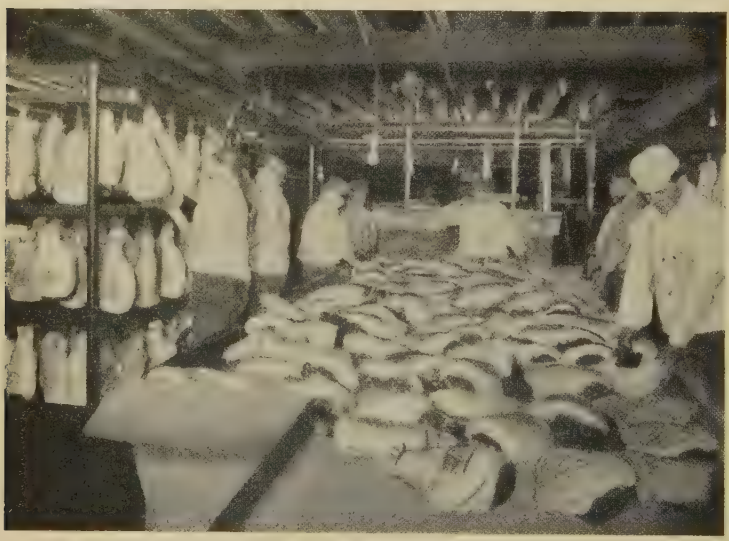


FIG. 85.—Preparing hams for smoking. (*Courtesy, Swift and Company.*)

onions are readily preserved by salting. Meats may be cured by either dry salting or in brine. Vegetables are placed in large tubs or tanks and covered with a brine containing from 12 to 25 per cent of salt. The salt does not kill all microörganisms, but the putrefiers and pathogens are retarded and in time probably destroyed. *Cl. botulinum* does not grow in brine containing over 10 per cent of salt. Often the surface of the liquid is covered with an oil to prevent the growth of molds and other aërobic microorganisms. Meats are usually dried to remove the excess of moisture on the surface in which microörganisms would multiply.

It is then smoked or various chemicals rubbed on the surface. These act as preservatives and prevent the microörganisms which may reach the meat from gaining entrance.

Sugar.—Fruits are often preserved by the use of large quantities of sugar which makes the osmotic pressure of the substance so great that bacteria cannot multiply in them. Molds are more resistant to osmotic changes, and are more likely to multiply in preserves than are bacteria. The molds are all aërobes; consequently, they do not multiply in the sealed containers. Grape sugar and levulose, which recent discoveries indicate may become a commercial product of importance, are more effective than is an equal weight of cane sugar. Salt and sugar are valuable means of preserving food as the salt can readily be removed by soaking, and the addition of water to the sugar preserved product returns the product to its normal digestibility, if not to its normal appearance and flavor.

Chemical Preservatives.—It is generally agreed that chemicals which act by concentration are not objectionable, whereas others may be. It is quite generally recognized that formaldehyde and many other chemical preservatives are injurious. The Federal Pure Food Law prohibits these from being placed in food which is to enter into interstate commerce. Authorities disagree on benzoic acid, benzoate of soda, and several other chemicals, some claiming that they are injurious, others that they are not. However, in the case of some food preservations the Federal Food Law requires that if these substances have been added they must be listed on the label. This permits the consumer to judge for himself whether or not he cares to use foods preserved by them. It is worth remembering that these chemicals preserve by inhibiting the action of the digestive enzymes of the bacteria, because they are actually protoplasmic poisons. Substances poisonous to bacteria are usually poisonous to man and the enzymes used by bacteria in their digestive processes are similar to those used by man; hence, if bacterial cells are to be injured or their enzymes rendered inactive by poisons, such preservatives will have similar action on human tissues and digestive juices. Moreover, chemical preservatives are especially vicious in the hands of unscrupulous manufacturers, for they make possible the use of products that could not be otherwise handled. They also make possible the handling of food by unsanitary methods.

CHAPTER XXVII

BACTERIA IN OTHER ARTS AND INDUSTRIES

Microbes have charge of life and death, of constructive and destructive changes. Some of these organisms are man's most destructive masters; others his most useful servants. Many are vital links in the chain which turns the wheels of life. They tear down the highly complex bodies of plants and animals from which the spark of life has departed and salvage the parts so they may be used again. They disintegrate waste products and change them into utilizable materials. Some microbes cause food spoilage often with disastrous results, whereas others are associated with the development of food flavors and at times even play a major rôle in food preservation. They manufacture alcohol, glycerol, acetic, butyric and lactic acid in addition to many other valuable products. They play a major part in the making of leather, the retting of flax, and in the curing of tobacco. In many changes they are the master chemists which initiate and control the reaction. Some of these processes have been known, although not understood, for ages; others are recent discoveries. Just a few of the most important ones can be considered in this chapter.

Silage.—There are approximately 500,000 silos in the United States, and the silage produced yearly runs into millions of tons. Any farm product can be siloed, provided it contains sufficient sugar to produce the requisite acid for its preservation. Although corn is more extensively used than other products, even sunflowers and weeds have been used and make an acceptable and nutritious food. The product is cut into small pieces and tightly packed into the silo. If it is too dry, water is added. On the surface of plants are millions of bacteria, the number varying with the material used. Minimum and maximum counts as reported by Löhnis are:

Green forage per gram	2,000,000 to	200,000,000
Hay per gram	7,000,000 to	17,000,000
Straw per gram	10,000,000 to	400,000,000
Grain (cereals) per gram	100,000 to	12,000,000
Concentrated feed oil cake, etc.	10,000 to	20,000,000

The living succulent plant tissues contain many active enzymes. While the plant is growing the microörganisms have no effect upon its tissues, and the enzymes are active in building new and repairing old tissues; but when the plant is chopped and packed into the silo the cell sap oozes from the injured parts. This mingles with the product and furnishes an excellent pabulum for the growth of microörganisms. The enzymes of the dying tissue which during the growth of the plant systematically built up, the tissues now commence to tear down. Within a few hours the oxygen is all consumed and the carbon dioxide content is changed from 0.03 to 18 per cent. After the oxygen is all used anaerobic changes occur, until by the end of the second or third day from 70 to 80 per cent of the silo gases are carbon dioxide. Occasionally a farm hand, ignorant of the gas, has been found dead in the silo, death having been caused by the lack of oxygen and not from a poisonous action of carbon dioxide. The changes which have occurred in the silage suppress some but favor other microörganisms. There is not sufficient oxygen to meet the requirements of the molds. The acidity retards the action of the yeast and putrefiers. Hence, acid producers rapidly multiply, and in a few days each gram of the silage may contain 2,000 million microörganisms. Sherman found from 1,500,000,000 to 4,800,000,000 microörganisms in each cubic centimeter of the silage juice. Most of these were slender rods and he considered them to be nearly related to the *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* groups.

FIG. 86.—Silo built of reinforced cement. (*Utah Experiment Station.*)

The numbers of bacteria present reaches a maximum and then declines, and by the end of three or four weeks relatively few living microörganisms remain. The predominating types which do remain are the streptococci and lactobacilli. Fermentation has almost ceased by the end of a month. The bacteria have done their work, and the enzymes have ceased to function. Heat

is no longer generated but is slowly radiated from the silage. Acids, alcohols, and carbon dioxide have been produced from the sugars. The plant tissues have been broken down and the entire mass has changed in appearance, flavor, and nutritive value. The material has been preserved and will keep for months or even years.

The changes which have occurred are great. From 10 to 15 per cent of the total dry matter has been lost. Most of this has gone off as carbon dioxide. Some may have been lost in the plant juices which occasionally drain from the silo. Much of the sugar has been transformed into acids. The quantity varies with the silage as may be seen from the following results reported by Neidig:

Silage from	Percentage acid in silage			
	Acetic acid	Propionic acid	Butyric acid	Lactic acid
Corn	1.8–4.0	0.2–0.3	0.0	4.0–6.1
Sunflowers	1.1–3.4	0.0–0.4	0.0–3.1	1.5–3.5
Oats and peas	1.5–2.2	0.1–0.2	0.0	4.6–5.0
Clover and straw	2.0–2.5	0.2	0.0	2.8–2.9
Alfalfa and straw	1.4–2.0	0.2–1.0	1.2–2.2	0.0

Alcohol is produced to the extent of about one-fourth of one per cent. The proteins are partly hydrolyzed. There is a decrease in the crude fiber. The ether extract increases, yet it is doubtful if there is any increase in fat. The green coloring material, chlorophyll, is changed to a brownish pigment.

The changes which occur in silage and the nature of the final product vary with the material siloed, the nature of the silo, and the temperature. Gorini distinguished four types of grass-silage prepared in pits, depending upon the predominating type of bacteria. They were butyric, lactic, putrefactive, and sterile or atypical. Butyric is likely to predominate where beet tops are siloed.

In heavily soiled material putrefactive and butyric acid changes usually predominate, and the resulting silage is very inferior or it may be a complete loss. It may also at times be dangerous. It is especially objectionable because of the odor and taste which

it is likely to impart to the milk. Moreover, the bacteria which enter from the surroundings render the milk unsuitable for cheese-making. Lactic acid predominates in silage properly made from corn and other products having a sufficient quantity of readily fermentable sugar. It is the ideal product.

Putrefactive changes predominate in silage made from heavily soiled material and materials high in proteins but low in sugars. If the silage stage reaches a temperature of 60° C. butyric acid organisms predominate; if 50° C., lactic acid organisms prevail; putrefaction occurs at lower temperatures, and sterile or atypical when the mass becomes super-heated. Attempts have been made in Switzerland to control the temperature by electrical means. The results are promising insofar as the quality of the product is concerned, but whether it warrants the extra cost remains to be seen.

Vinegar-making.—The making of vinegar represents two steps, each brought about under definite conditions by specific micro-örganisms. The first is the production of alcohol by micro-örganisms, usually yeast acting on a sugar. Any material which contains from 12 to 16 per cent of sugar, such as fruit juice, malt extract, diluted honey, or molasses, is suitable raw material for alcohol production. Although alcoholic fermentation usually will occur due to organisms which are on the product, the best results are obtained by seeding with a suitable culture of yeast. If the alcoholic liquor is produced from fruit or grain extracts which have been decomposed by foreign bacteria, neither the alcoholic fermentation, nor the acetic acid fermentation is normal; hence, the resulting vinegar has off-flavors. The rate of the reaction varies with the temperature. The optimum lies between 22° and 26° C. The time required for the completion of the alcoholic fermentation in the ordinary farm process where the freshly pressed fruit juice is placed in casks and allowed to stand at a temperature of from 7° to 13° C. is six months. Alcoholic fermentation should be complete before the starting of the acetic acid fermentation as yeast does not work well in the presence of acetic acid.

The second step in the manufacture of vinegar is an oxidation process. It is brought about by the action of the acetic acid bacteria upon the alcohol which is caused to take up oxygen and become acetic acid. Ordinarily, the vinegar bacteria if undisturbed form a film on the surface of the alcoholic solution. This is known as the "mother of vinegar." When Pasteur investi-

gated the troubles of the vinegar makers of Orleans, he found that they did not appreciate the great importance of supplying their fermenting mixtures with plenty of oxygen. In order to provide this, he made more openings in the casks and devised a raft of slats. This was placed on the surface of the liquor and acted as a support for the mother of vinegar."

There are two methods in general use for the manufacture of vinegar from alcoholic liquors: the Orleans, and the quick or German methods.

The Orleans Method.—This is the oldest commercial method and produces vinegar of the highest quality. However, it has the disadvantage of being cumbersome, laborious, slow, and costly. Then too, there is a loss of about 10 per cent of the material by evaporation, and the repeated additions of liquid break the bacterial film which sinks to the bottom and grows anaërobically exhausting the nutrients without the production of acetic acid. There are many modifications of the method, but they are all based essentially on the same principles. The filtered wine is placed in barrels or covered vats furnished with openings so that the entrance of air is facilitated and can be controlled. The receptacle is filled about two-thirds full of a mixture of four parts of good, new vinegar, and six parts of wine, preferably that which has been pasteurized at 55° C. A small quantity of a good bacterial film is put into this solution as a starter. Periodically a portion of the contents is drawn off and replaced by wine and so the process continues. Pasteur changed to shallow vats with regulated air vents instead of barrels or vats, and on the top of the fermenting liquor he placed a perforated wooden float to support the "mother of vinegar." Acetic acid bacteria will grow slowly in a weakly acid or even slightly alkaline medium, but a more prompt and vigorous growth is obtained in a decidedly acid medium. For this reason, vinegar is added to speed up the action of the acetic acid bacteria and to prevent the growth of undesirable microörganisms.

The Quick Vinegar Process.—In this method large wooden tanks with perforated bottoms are used. These are filled with beech chips, the pressed pomace of red wines, rattan shavings, corn cobs, or charcoal. Although the main function of these is to increase aëration, the best results are obtained with beech shavings, or pomace, for they impart no undesirable flavor or color to the product. The solution to be acidified is automatically sprayed on the surface of the shavings and slowly trickles

down in a continuous film. This is acted upon by the acetic bacteria that have previously been seeded on to the shavings, and the heat generated causes a constant draft which furnishes the microörganisms with the requisite oxygen.

On standing for some time vinegar may lose its strength because of the action of certain bacteria (*Acetobacter xylinus*) which in the presence of oxygen split the acetic acid into simpler compounds. To avoid this, the finished product is pasteurized

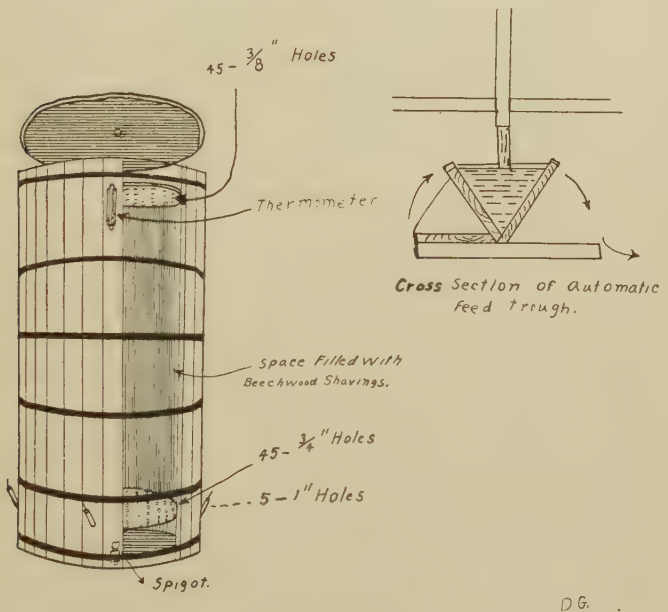


FIG. 87.—Drawing of barrel used in vinegar making.

or stored in air-tight containers. Pasteurization is believed by some to assist in the maturing of vinegar, giving to it a softer and more pleasant taste and aroma. This is thought to be due to the hastening of the reaction of the residual alcohol and the acetic acid with the formation of esters.

Curing of Tobacco.—During the curing period, tobacco undergoes profound changes. The texture and aroma are greatly influenced. There is about a 28 per cent loss of nicotine and a gain in citric acid. These are fermentative changes caused by enzymes and microörganisms. Some investigators have made

studies in the laboratory in which they have used pure cultures and obtained what they considered to be a superior product with a more satisfactory aroma than is obtained when the tobacco ripens under natural conditions. The bacteria found in the curing of tobacco seem for the most part to belong to the proteus, subtilis, and mycoides groups. Other investigators have obtained equally good results in curing tobacco in the presence of disinfectants which prevented the actions of microörganisms but did not interfere with enzymic action. Others have found that as the temperature of the fermenting tobacco increases, it gradually becomes sterile. Löew holds the idea that the curing of tobacco under natural conditions is not essentially a bacterial process but due to leaf enzymes, oxidases, peroxidases, and catalases. Probably both enzymes and microörganisms play a part, but at the present time it is impossible to state which are the most important. A British patent has been taken out for the improving of inferior or ordinary tobacco and giving to it a flavor resembling Havana and other superior qualities. The material is treated with liquid cultures of one or more of the following organisms: butyric ferments, aromatic lactic-acid bacteria, ester-forming species of torula, and ester-forming fungi. The cultures may be grown in various liquids such as malt extract, milk whey, and solutions of different sugars along with decoctions of various aromatic vegetables, and this applied to the tobacco or the cultures; or the unfermented material may be added and the fermentation permitted to occur on the tobacco.

Cocoa.—Cocoa and the various chocolate and cocoa preparations are made from the beans of the cocoa tree, *theobroma cocoa*. The beans grow in pods, varying in length from 23 to 50 cm., and are from 10 to 15 cm. in diameter. The beans which are about the size of almonds are closely packed in the pod, and are white in color when fresh until they are dried, when they turn brown. These beans are mixed with plantain leaves and placed in barrels. The sweet outer covering of the bean immediately commences to ferment and the mucous covering is liquefied. Heat develops due to the fermentation. After about four to seven days the seeds are taken out, spread on trays, covered, and left for another day to complete the process. Löew says: "The process of fermentation depends especially on yeast cells which increase rapidly in the syrup of the cocoa bean, with the formation of alcohol and carbon dioxide. Also bacteria take part in the fermentation, oxidizing the alcohol to acetic acid. A rise in temperature takes

place and the slimy tissue surrounding the seeds comes off and collects on the bottom of the vat. The removal of this tissue is the chief end of the fermentation. It causes the seeds to be more easily dried. An oxidation of the tannin takes place, which causes the brown color of the bean. The taste and the aroma depend on the fermentation as well as on the roasting."

Retting of Flax and Hemp.—"Hemp was probably the earliest plant cultivated for the production of a textile fiber. The 'Lushi' a Chinese work of the Sing dynasty, about 500 A. D., contains a statement that the Emperor Shen Mung in the twenty-eighth century B. C. first taught the people of China to cultivate 'Ma' (Hemp) for making hempen cloth." Hemp makes a coarser fabric than does flax, but the fiber from both plants is separated by a process of retting or rotting. This may be carried out either by dew-retting or water-retting. "In this country dew-retting is practiced almost exclusively. The hemp is spread on the ground in thin, even rows, so that the butts are all even in one direction and the layer is not more than three stocks in thickness. Warm, moist weather promotes the retting process, and cold or dry retards it. Hemp rets rapidly if spread during early fall, provided there are rains, but it is likely to be less uniform than if retted during the colder months. It should not be spread early enough to be exposed to the sun in hot, dry weather. Alternate freezing and thawing or light snows melting on the hemp gives most desirable results in retting. Slender stocks one-fourth inch in diameter or less ret more slowly than coarse stalks, and such stalks are usually not over-retted if left on the ground all winter. Hemp rets well in young wheat or rye, which holds the moisture about the stalks. In Kentucky most of the hemp is spread during December. A protracted January thaw with comparatively warm rainy weather occasionally results in over-retting. While this does not destroy the crop, it weakens the fiber and causes much loss. When retted sufficiently, so that the fiber can be easily separated from the hurds or woody portion, the stalks are raked up and set up in shocks, care being exercised to keep them straight and with the butts even." "Water-retting is practiced in Italy, France, Belgium, Germany, Japan, and China, and in some localities in Russia. It consists in immersing the hemp stocks in water in streams, ponds, or artificial tanks. In Italy where the whitest and softest hemp fiber is produced, the stalks are placed in tanks of soft water for a few days, then taken out and dried, and returned to the tanks for a second ret-

ting. Usually the stocks remain in the water first about eight days and the second time a little longer.

"In either dew-retting or water-retting the process is complete when the bark, including the fiber, readily separates from the stalks. The solution of the gums is accomplished chiefly by certain bacteria. If the retting process is allowed to go too far, other bacteria attack the fiber. The development of these different bacteria depends to a large extent upon the temperature. Processes have been devised for placing pure cultures of specific bacteria in the retting tanks and then keeping the temperature and air supply at the best for their development. These methods which seem to give promise of success have not been adopted in commercial work."

The fermentation process consists essentially in dissolving the cementing substance known as pectins which hold the bast fibers to the stock. In the water-retting of hemp this is attributed to the anaërobe *B. amylobacter*, whereas the water-retting of flax is attributed to the anaërobe *Granulobacter pectinovorum*.

In Belgium and Holland where much of the finest retting is done, it is stated that certain streams are better adapted to the retting process than are others. This may be due to a variation of the microflora of the various streams, as it is likely that some organisms are better adapted to carry on the process than are others.

Efforts have been made to liberate the flax fiber by means of chemicals, but none of these efforts have been fully successful as it is found that chemicals strong enough to dissolve the pectin also weaken the flax fiber and destroy its luster.

The Tanning of Hides.—The making of leather is an art, the history of which extends into antiquity. Many half-civilized people knew how to tan hides. The American Indian was an expert in the making of leather from many hides. "The leather industry of today is an evolution covering many centuries. It is a far cry from the crude primitive methods employed in the earliest historic period to the highly complex process of the modern tannery. Probably the first method of curing skins was simply cleaning and drying. Then it was found that the use of smoke, sour milk, various oils, and even the brains of the animals from which the hides were stripped improved the quality and texture of the leather; still later it was discovered that certain astringents, barks, and vegetables effected permanent changes in the texture of the skins and stopped decay. The use of bark and vegetable

juices has continued and forms the basis of many of the modern tanning processes. The Egyptians possessed this knowledge, for pictures and hieroglyphics describing the methods are found on many of the ancient tombs in the Nile Valley. Three thousand years ago the Chinese were familiar with leather preservation, as various relics handed down from antiquity serve to show. Leather preserved and tanned with oil, alum, and bark was also used by the Romans."

The various steps which enter into the making of leather from hides may be grouped under the several heads: (1) The preservation of the hides; (2) disinfection; (3) the soaking and fleshing; (4) depilation; (5) batting; and (6) the final tanning process. The transformation which occurs in many of these processes, especially in the early methods of tanning, is brought about by microbial action.

Although the inner surface of the skin as it comes from the animal is free from bacteria, it rapidly gathers them from various sources. These if permitted would multiply rapidly and under appropriate conditions destroy the skin. To prevent this the green hide is treated with salt; this reduces the water content of the skin from 65 to 45 per cent. It also stops the action of bacteria on the proteins of the skin and at low temperatures as long as the solution wetting contains 6 per cent salt, there is little danger of putrefaction. If the strength of the salt solution falls below one per cent it favors putrefaction. The process of salting is known as curing.

Because infectious diseases have been spread by infected hides it is the custom to disinfect some hides. This is done by soaking them in a 2 per cent solution of mercuric chloride containing 1 per cent formic acid, or a 10 per cent solution of salt in 2 per cent hydrochloric acid. This is especially necessary in the case of hides which have come from animals infected with anthrax which is pathogenic to man and may survive the various tanning processes.

On reaching the tannery, the hides are first soaked to render them pliable and to permit bacteria to remove the excess of tissue which adheres to them. In the old process bacteria were depended upon to remove most of the excess flesh, but today this is accomplished primarily by passing the skin over revolving rollers on which are knives which cut away the excess tissue. Bacterial counts are occasionally made of the soak water so as to guard against excessive numbers.

Following the soaking comes the depilation—the removal of the hair. This is accomplished in a number of different ways such as liming, sweating, use of enzymes, acids, or alkalies. The earliest method of removing the hair from hides was by means of incipient putrefaction. This causes the soft mucous matter of the epidermis to become affected, and the hair to become loosened without materially injuring the true skin. This method is still employed by many manufacturers of sole leather and is called sweating. The operation is conducted in closed rooms which are

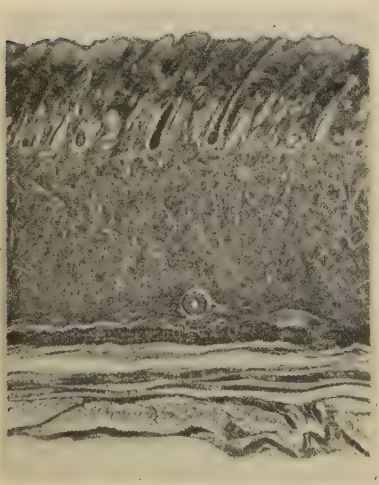


FIG. 88.—Vertical section of calf skin (fresh) magnified 30 diameters. (*Courtesy, A. F. Gallun and Sons Company.*)

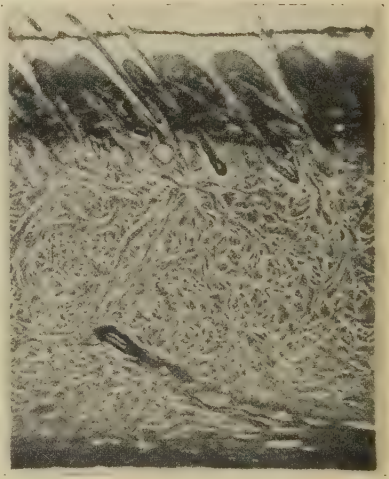


FIG. 89.—Vertical section of calf skin (after 48 hours in lime liquor) magnified 35 diameters. (*Courtesy, A. F. Gallun and Sons Company.*)

kept at a temperature of 70° F. The hides are hung in small chambers ("sweat pits") holding about 100 hides each. A large quantity of ammonia is given off during the decomposition which aids in the solution of the epidermis and the loosening of the hair. After four to six days of this treatment the hair is sufficiently loosened to be removed by working over a rounded beam with a blunt knife made for the purpose. Hides which have been unhaired in this way are soft and fallen and must first be swollen by placing in an acid liquor before entering the tanyard. This treatment is especially necessary in the case of sole leather. However carefully this operation is conducted, there is liability

that putrefaction may attack the skin itself, thus, causing a weak grain. For this reason this method has been to a great extent replaced by the liming process. The hides are soaked for some time in a saturated lime solution. The lime causes the hide to swell, softens the epidermal cells, dissolves the mucous layer, and loosens the hair so that on scraping with a blunt knife, both the epidermis and hair are easily removed. The solution increases in efficiency with use. This is thought to be due to the bacteria which accumulate, as the medium soon contains thousands of proteolytic ferments. Wood has isolated 90 species of bacteria from such a vat, none of which possessed the power of bringing about the desired change, but all acting conjointly produce the desired product.

The lime is removed and the material is brought from its swollen to a soft and open condition by placing in a weak fermenting infusion of pigeon or hen manure. The acids remove the lime salts. The process is called batting. The product is next transferred to the tan pile or into vats containing the tan liquors. In some of these processes microörganisms play a major rôle in finishing the product.

CHAPTER XXVIII

AIR

Common air is at the basis of our very existence. From air we obtain our oxygen supply, and indirectly through the plants carbon, hydrogen, and nitrogen. Air controls the light, temperature, and moisture of our surroundings and brings to the plants their required food. We could live for weeks without food and for days without water, but only a few moments without air. Air is the cheapest, most abundant, and most essential of the three. Everyone desires a pure water; some give considerable attention to the quality and quantity of the food they consume, but many are quite indifferent as to the nature of the air they breath.

Composition.—The atmosphere at or near the surface of the earth is composed of 78.1 per cent by volume of nitrogen, 20.9 per cent by volume of oxygen, and 0.9 per cent by volume of argon. Carbon dioxide is normally present to the extent of 0.03 per cent, and the inert gases other than argon (neon, helium, xenon, krypton) in still smaller percentages. Air also carries varying quantities of moisture, dust, soot and microörganisms.

Microörganisms in Air.—For normal growth and reproduction all bacteria require water, food, a suitable temperature, and usually the absence of direct sunlight. The moisture conditions of the atmosphere at times may be optimum, but generally none of the other conditions are; hence, soil, water, and milk have a natural microflora, but air has not.

The microörganisms in the atmosphere vary from place to place and from day to day. They enter the air from the surroundings. The bacterial content of dust varies from a few thousand to many million in each gram. The hurrying feet, rapidly moving wheels, and the ever-acting currents of air carry them into the atmosphere. The spray from the beating surf, the droplets from the mouth of the speaking, laughing multitudes, all add their quota,—in short,—anything which increases the suspended particles in the atmosphere usually increases its microbial content. The exception is where antiseptic substances are finding their way into the air. For example, the air of Lon-

don streets contains between 300,000 and 500,000 solid particles per cubic centimeter, but there is only one microörganism to every 38,200,000 dust particles.

Bacteria are most plentiful in the air of densely populated areas. They are especially numerous in some factories, churches, theatres, dance halls, and other places where large numbers congregate. Miquel found that on an average one cubic meter of air from the streets of Paris contained 3,480 bacteria; laboratory air, 7,420; and the air of old houses 79,000. Winslow and Browne found one foot of air from the country to contain 56 microbes; city street air, 72; office and school air, 95; and factory air, 113. They found streptococci (characteristic of specific mouth pollutions) in outdoor air to the number of 11 to 12 per 100 cubic feet; in office air, 22; school air, 30; and in factory air, to the number of 43. The number of microörganisms in the air also varies with the distance from the earth at which the air is collected. At the top of the clock tower of the house of Parliament in London Graham Smith found only one-third the number of bacteria that he found at ground level. Whipple found 1,330 bacteria per cubic foot in air at street level, whereas at the tenth story of the John Hancock Building the air contained only 330. Furthermore, the classic experiments of Pasteur showed the air of streets and workshops to contain many microörganisms, whereas the air over the surface of glaciers and on the tops of high mountains was often free from them.

Factors Governing Number in Air.—Moisture greatly increases the number of bacteria in soil, but it decreases the number in the air, for it moistens the particles of dust, causes them to settle out and to carry with them the bacteria. The air of the country contains fewer bacteria than does the air of the city. This is due in a measure to the larger quantity of organic matter in the dust of the city, and also to the numerous ways in which bacteria are being carried into the air.

On mountain tops, over deserts, and in other uninhabited regions the air is nearly free from bacteria. The classic illustration of this fact is found in the experiments that Pasteur conducted in his efforts to refute the doctrine of spontaneous generation. Of twenty flasks exposed to normal country air at Arbois, eight showed the presence of microörganisms, whereas a similar series of sterile flasks opened on the slopes of the lower Jura mountains only five spoiled; and of a third series exposed on the Mer de Glace, only one became infected.

The number of bacteria in air over oceans is low and varies with the nearness to land. Close to shore there are often very many, whereas at great distances from land the air may be free from microörganisms.

The number of microörganisms in air decreases with the altitude as well as locality. Jean Binet did not find a single microbe in 100 liters of air taken on the summit of Mount Blanc. The number rapidly increased on decending.

At the Summit	0
At the Grand Plateau	6
At the Grand Malet	8
At the Place de l'Aiguille	14
At the Mer de Glace	23
At Montanvert	49

The numbers vary with the season, increasing from winter to summer and decreasing from summer to winter. There is also a marked decrease in the number of bacteria in the air after a rainstorm. The rain carries them to the ground and also moistens the surface so that particles of dust are not carried into the air by every breeze. However, the added moisture of the soil greatly increases the speed of multiplication, so that later as the surface soil dries out, more dust and with it a greater number of bacteria are carried into the air. It is also true that the number of microörganisms in the air decreases in the winter months not because cold is inimical to the life of the microörganisms, for just the reverse is true, but because the conditions are not as good for them to find their way into the atmosphere. This is due to the fact that the soil is covered with snow or with considerable moisture which prevents the dust from being carried into the atmosphere.

It is quite evident that there would be a relationship between the number of bacteria in the atmosphere and the climate of that region. Bacteria multiply rapidly in the soils of a warm, humid district and find their way into the atmosphere where they remain until they are washed out by rain. There is, as one can readily see, a great variation in the bacterial content of the air of warm moist regions. In arid places the number in the air may be smaller but does not vary as greatly as in the more humid sections.

Bacteria remain in the atmosphere for varying lengths of time. Their stay depends on several factors:

1. The hardy spore-forming saprophytes may remain suspended in the air for days or even weeks, whereas the frail non-spore-forming pathogens soon perish due to either drying or the sterilizing action of the sun's rays.

2. Small particles settle out more slowly than do large ones, for as the size of an object is decreased the surface area decreases less rapidly proportionately than does the volume. Hence, these bacteria which are floating free in the atmosphere settle out more slowly than those attached to dust particles.

3. The time of suspension is also determined by the velocity of the air current. Microörganisms settle out of a still atmosphere more readily than from one in motion. It may require an air current of considerable velocity to dislodge microörganisms and bring them into suspension, yet a slight current will sustain them.

4. Moisture in the atmosphere tends to cause particles to adhere together, and as they grow in size the tendency for them to settle out is proportionately increased.

5. Although the air of London and many other large cities contains numerous particles of dust, the number of living organisms is comparatively small, for the various gases thrown into the atmosphere have a slight germicidal effect upon the bacteria.

Bacteria in Inspired and Expired Air.—The number of bacteria inhaled by man depends on the number of microörganisms in the air. It is estimated that a person living in London breathes about 300,000 bacteria each day, and individuals living in other districts may take in many times this number. Most of these bacteria are harmless and are caught on the moist mucous membranes of the upper respiratory passages, only very few finding their way into the deeper alveoli, and the majority of those that do are quickly destroyed by the phagocytic cells of the body.

It has been shown that the expired air is entirely free from germs. The reason for this is that they are not thown off from moist surfaces. Even a considerable current of air fails to dislodge them. The expired breath of an individual suffering from diphtheria, tuberculosis, or any of the other respiratory diseases is entirely harmless. Coughing, sneezing, or forceful speaking may dislodge them and cause them to be thrown off in a fine spray, but this does not occur in normal, or even deep breathing.

Droplet Transference of Bacteria.—Flügge was the earliest investigator to call attention to the fact that during speaking, and especially during loud talking, coughing, and sneezing, tiny

droplets of saliva are thrown into the air. Anyone can observe the fine spray coming from the mouth of individuals standing in certain lighted positions during these actions. In order to test the extent to which this spray may carry bacteria into the air, various investigators infected their own mouths with *Erythrobacillus prodigiosus* and then read, spoke, coughed, and sneezed in rooms in which cultural dishes were exposed. These dishes collected and indicated the presence of the organism. In some cases, it was found that the germ-laden droplets expelled during coughing were carried to a distance of nine meters from the mouth, and in a few cases to the distance of two meters behind the person coughing. The comparative results as obtained by various investigators are tabulated below.

	Total plates exposed	*Erythrobacillus prodigiosus* figures indicate colonies per person	Percentage of positive plates
Laschtschenko	36	17.2	83
Koeniger	331	5.2	61
Gordon	30	0.7	53
Winslow and Robinson	325	2.4	48

Some have interpreted these results as indicating that a general infection of the atmosphere takes place over a wide radius and that there is a real danger from breathing the air in the neighborhood of an individual ill of any of the respiratory diseases. This is more apparent than real, for Koeniger showed that 60 per cent of the bacteria disappear from the air in ten minutes, while after 20 minutes less than 10 per cent of the original number remain.

Where attempts were made after speaking, coughing, etc., to detect organisms in the air which are normal inhabitants of the mouth it became evident that the results obtained in the artificial infecting of the mouth were far too high. In quantitative tests of the air itself at 35 cm. to 2.4 meters in front of speakers who spoke vigorously in English or German. Mouth streptococci usually failed to be present in large volumes of air. The result is that Flügge's own treatment of the matter is considered conservative by most health workers today. He pointed out that in experiments carried out by his own pupils, tubercle bacilli were abundant only within 0.5 meter of the coughing patient and that

beyond 1.5 meters their numbers were so small as to make the chances of infection practically nil. The rule laid down by him is the best yet formulated for the prevention of the spread of the respiratory diseases: "During strong paroxysms of coughing, the consumptive should keep at arm's length from his companions and should hold a handkerchief before his mouth. In work rooms, offices, and such places the space between the workers should be at least one meter."

Air-borne Infection.—The theory that miasmatic vapors in the atmosphere are the cause of disease dates back to the times of Hippocrates when fires were lighted in the streets of Athens to purify the air and put a stop to the plague. Such a belief was only natural, for the viruses of the communicable diseases were believed to be gaseous, or at least readily diffusible substances borne by air currents. And what was more reasonable than to believe that these got into the air from the breath of the patient coming as the breath does from the very vitals? Night air was considered to be especially bad, for it was believed to carry malaria to the individual. The formulating of the germ theory of disease strengthened this belief in the minds of many. Germs of disease are often found in the nose and throat of the sick, and it seemed reasonable to believe that such tiny particles could easily find their way into the air where they might float for some time. The work of Pasteur demonstrated that in so far as the decomposition of organic material was concerned the germs carried by air play a predominant rôle. When Lister in 1867 devised his first antiseptic spray, it was the microbes of the air which loomed largest in his mind as potent factors in wound infection.

The first shock to this belief came when it was shown and proved by experiments that the expired air is sterile. Then too, the careful quantitative work of Winslow and others showed that pathogens forced from the mouth and nose in the acts of speaking, coughing, and sneezing travel only short distances in the droplet, and that when dislodged from the droplet soon perished because of the action of the oxygen of the air, light, and the loss of moisture. Of the numerous careful epidemiological studies that have been made of various diseases, all have yielded negative results. The work of the surgeon has clearly demonstrated that even the widely distributed pyogenic organisms get into the wounds not from the air but from the skin of the patient, or the instruments which produced the wound, or other objects which came in contact with it. Moreover, it is the experience of well-managed

hospitals that various communicable diseases may be kept in a single open ward without serious danger of cross infection, provided care is taken to prevent contact infection. Even the malaria, yellow fever, and the bubonic plague, which seemed to settle from the air on whole cities at a time, are now known to be conveyed by insects, and not by air; and it was the conclusion of the Influenza Commission after its study of the great influenza epidemic which so quickly spread over all quarters of the globe, that this disease, also, travels in a "pair of shoes" and not in the air. Hence, today it is the consensus of opinion among health authorities that this spectre of air infection, which has plagued the race from the times of Hippocrates, plays little if any part in the transference of disease. Today efforts are used to prevent contact infection and not air infection.

Odors and Health.—It has long been a popular belief that foul air not only causes illness but often gives rise to communicable diseases. Leading sanitarians taught from 1880 to 1890 that sewer gas gave rise to diphtheria and other diseases. The laws concerning nuisances as they exist today in many cities and states are based on this belief. Careful examination of the gases emitted from sewers and cesspools failed to reveal the pathogens. Today it is known that sewer air, unless there be a splashing and particles are mechanically thrown into the air, is as free of pathogens as is the east wind, and consequently cannot give rise to communicable diseases.

Coal gas, illuminating gas, chlorine, hydrogen sulfide, and other chemical gases may injure health, or even cause death as a result of their direct poisonous action. Lower animals as well as man do best under pleasant agreeable surroundings; hence, it is not surprising to find, as Winslow did, that guinea pigs exposed to the odor of putrefying organic material showed a less rapid growth curve for the first few days of exposure than normal animals. However, the effect was only temporary and the animals later caught up with the controls. The New York Commission on Ventilation found that offensive odors which came from improper ventilation and putrefaction had two slight but distinctly measurable effects upon human behavior. Subjects exposed to stale air showed a definite disinclination to physical activity and accomplished 9 per cent less work in stale than in fresh air. The effects also manifested themselves in the appetite of the individual, for the amount of food eaten at luncheon by those who were kept in fresh air was from 4 to 14

per cent more than that eaten by those kept in stale air. The subject is summarized by Winslow as follows: "We may, therefore, conclude that while air does not as a rule bear the germs of disease and does not carry with it mysterious and specific organic poisons, an atmosphere laden with offensive odors is not only objectionable on esthetic and economic grounds, but may under certain circumstances constitute a real menace to the public health."

Ventilation.—Many changes occur in the air of an unventilated room as a result of human occupancy. These may be grouped under five heads: (1) The oxygen content of inhaled air is 20.9 per cent by volume, whereas that of exhaled air is only 16 per cent. A man of average weight, at rest, consumes about 534 liters of oxygen in twenty-four hours. (2) Inhaled air contains 0.03 per cent by volume of carbon dioxide and exhaled air 5 per cent. Under average conditions of rest an individual will produce about 427 liters of carbon dioxide in twenty-four hours. (3) There is given off into the air from the body in the breath, prespiration, and from the clothing a variable amount of organic substance which gives to the air the "stuffy odor" which one notices on entering a poorly ventilated room. (4) The temperature of the air is raised due to oxidations occurring in the human body. As an average for each individual, the heat liberated amounts to approximately 2,500 calories in twenty-four hours. (5) The humidity of the air is increased due to the moisture given off in the breath and prespiration. The amount is variable, but the contribution is about 1,400 c.c. in twenty-four hours. If the individuals are engaged in physical activity these quantities may be doubled, trebled, or even quadrupled.

In a crowded, ill-ventilated room one experiences a feeling of lassitude, dullness, sleepiness, and if continued long enough there results nausea and faintness. There are cases on record in which it has been carried to such an extent that death has resulted.

The effects have been attributed by various workers at different times to the following changes; (1) a loss of oxygen; (2) an increase in the carbon dioxide; (3) poisonous organic substances given off from the body; and (4) temperature and moisture changes which have occurred in the air.

The effect cannot be due to a diminution of the oxygen content, as even in the worst ventilated rooms oxygen never falls below 20 per cent, and it is only when it falls from its normal of 20.9 to 16 per cent or less that important physiological reactions occur.

Individuals often live at altitudes, and in reality some of them are known for their beneficial effects on health, where the oxygen pressure is far lower than it ever becomes in poorly ventilated rooms.

The ill effects of an atmosphere vitiated by human beings cannot be attributed to an increase in the carbon dioxide content, for even in the poorest ventilated rooms it is unusual to find as high as 0.5 per cent carbon dioxide. The air of breweries has been found to contain from 1.5 to 2.5 per cent carbon dioxide, yet men have worked continuously in these without any ill effects. Moreover, numerous experiments have definitely proved that an atmosphere may carry as high as 3 per cent of carbon dioxide without producing any measurable ill effects upon human beings.

It is not probable that the ill effects are caused by a poison exhaled in the breath, for repeated search for such a substance has yielded only negative results. Hence, it is today generally believed by health workers that "the physiological problems of ordinary ventilation have ceased to be chemical and pulmonary, and have become physical and cutaneous." The work of recent years has shown that all the effects of poor ventilation can be reproduced under experimental conditions by overheating, excessive moisture, and lack of air movement. Subjects have been placed in experimental chambers and kept there while the products of respiration accumulated. The subjects eventually manifested all the symptoms of poor ventilation. Various means were then used to relieve the symptoms. They were permitted to breathe fresh air through tubes connected with the outside while their bodies were bathed in the vitiated air of the chamber. This did not relieve the symptoms due to poor ventilation. However, subjects were completely relieved when the air of the experimental chamber was cooled even though they were breathing the same foul air which a few moments before was causing them such distress. In other tests the carbon dioxide was permitted to accumulate to such an extent that a match would not burn and the distress became very pronounced, yet the subjects were all relieved when the air was set in motion by means of a fan. In still other tests people outside were permitted to breathe from the vitiated experimental chambers through a mouthpiece while their bodies were bathed with the fresh cool air of the outside. They felt no discomfort. These same individuals immediately became uncomfortable on entering the

experimental chamber. Hence, the conclusion has been reached that the feeling of discomfort and lassitude experienced under poor ventilation is probably due directly to anemia of the brain and other internal organs caused by the pooling of the blood in the capillaries. This manifests itself by a measurable increase in body temperature and a lowered immunity which may persist for hours after the condition which caused it is relieved. The far-reaching effect of excessive temperature is thus summarized by Winslow: "A temperature in excess of 68° F., with little air movement and moderate humidity; exerts direct and important effects upon the circulation, causing particularly an increase in body temperature, a rise in heart rate, and a fall in vaso-motor efficiency; it profoundly limits efficiency by causing a disinclination to physical exertion; and it markedly increases susceptibility to disease." For a room to be properly ventilated the air coming into it must be free from dust and injurious fumes; kept in slight but not observable motion, have a humidity of approximately 50 per cent, and a temperature of from 62° to 68° F. The temperature must vary with the occupant; very young, old, or ill individuals will require a higher temperature than do others. Individuals engaged in physical activity require a lower temperature than those engaged in sedentary work. It is evident from this that the thermometer is essential in ventilation, and "no school room, no factory work room, no office, no living room should be considered as furnished and fit for human occupancy without a thermometer." This is stressed by a New York City regulation requiring that a large thermometer, with a heavy red line opposite 68° F., be placed in a conspicuous place in every school room.

CHAPTER XXIX

THE INTESTINAL BACTERIA

We are living in a world teeming with bacteria. They are on everything we touch. Practically every bit of food and every drop of water we consume contains few or many bacteria, depending on the past history of these foodstuffs. What is the fate of the microbes after they enter the alimentary canal? Are they killed? Do they multiply or remain dormant? May they be reduced in numbers or replaced in kind? Do racial dietary habits influence the microbial germs? Do they help or do they hinder? Is man what his microbes make of him? Alcohol is a microbial product which, if given to man in small quantities is a stimulant, in larger quantities a depressant, and in large quantities it may be a fatal poison. This is true of many poisons, including those produced by pathogens. Do the saprophytic and parasitic microbes which swarm in the alimentary tract of man produce stimulants, depressants, or highly potent poisons? Each specific plant produces its peculiar fruit; one does not expect to "gather grapes of thorns nor figs of thistles," consequently, the products vary with the microflora.

Number.—The mouth is a microbial garden, the number and variety of microbes varying with each individual. The well-kept mouth, free from disease and containing a set of healthy teeth may contain few; whereas the mouth filled with decaying teeth each cavity and crevice filled with decaying and putrefying food contains many. The bacteria of the mouth, together with those in the food and water, are carried into the stomach. Here they meet the acids of the gastric juice which stop all multiplication and destroy most of the least resistant microörganisms. Hardy spores, microörganisms imbedded in masses of food, and those which reach and leave the stomach when acid secretions is in abeyance, or when the acid is neutralized by proteins find their way into the intestines. Here conditions of food, warmth, moisture, and reaction are ideal for the microbial growth. Relatively few bacteria pass the stomach, and the sojourn in the duodenum is comparatively short; hence, the microflora of this

part of the intestines is sparse. Cushing and Livingston have called attention to the phenomena that gunshot wounds in this region are far less likely to develop peritonitis than are wounds lower down, for only few bacteria enter the abdominal cavity from duodenal wounds. This in a measure, accounts for the difference. From the duodenum to the ileocecal valve the number of bacteria increases rapidly, but the maximum is probably not reached until the ascending colon. Beyond this, the number of living bacteria rapidly decreases, for the feces contain approximately 90 per cent dead bacteria. The numbers excreted daily by a healthy adult on a mixed diet have been variously estimated from one hundred to thirty-three hundred billions. This number of dry bacterial bodies would weigh more than five grams and would contain about 0.6 gram of nitrogen This is approximately 42 per cent of the total fecal nitrogen.

Kind of Bacteria.—It is evident that the enormous number of bacteria excreted daily cannot represent those taken in with the food which really contains relatively few, but rather indicates that rapid bacterial multiplication occurs in the alimentary canal. The kind of microörganisms and rapidity of multiplication vary in different individuals, depending largely upon the nature of the food.

The alimentary canal of the child at birth is sterile. During the first few days, which Kendall refers to as the period of "adventitious bacterial infection," the child obtains various microörganisms from the air and the things with which he comes in contact. These bacteria however, are only temporary inhabitants of the intestines and are soon replaced by others. The kind that eventually becomes established depends very largely on the food of the child.

The upper part of the small intestines of breast-fed children contains *Enterococcus*, *Streptococcus lacticus*, and a general predominance of the coccoid form. Lower down and before the ileocecal valves, the *B. lactis aërogenes* and the *Bact. coli* appear. In the lower part of the colon and the rectum, *B. bifidus* and similar anaërobes predominate. Many proteolytic bacteria may be also present. In the upper bowels of the artificially fed child occur members of the colon and aerogenes groups, and considerable numbers of *B. mesentericus* and other anaërobic spore producers; but in the lower bowels are large numbers of the *Lactobacillus acidophilus* and related organisms.

As adult life is attained greater numbers of *Bact. coli* and *B. mesentericus* occur along with several species of gas producers. Often *Bact. coli.* constitute 75 per cent of the bacterial flora. The alimentary canal of an individual on a mixed diet, especially if it is rich in protein, contains putrefiers *proteus vulgaris, B. aërogenes capsulatus,* and *B. putrificus.* These organisms are supposed to be associated with the production of certain toxic substances which give rise to so-called "autointoxication." However, this has not yet been proved. In the presence of excessive quantities of carbohydrates a diarrheal condition may be caused in children by the irritating products produced in *Cl. welchii.* According to Jordan many instances of anemia in children and adults seem to be accompanied by a chronic infection of the intestinal tract with this organism, and as the general condition of the patient improves there is a distinct reduction in the numbers of this organism that can be found in the feces.

The relationship of the host to its intestinal microflora is summarized by Kendall as follows: "The prominent types of bacteria that appear in the intestinal flora of a normal person are rarely constant in their occurrence, but there may be a well-marked seasonal and even annual variation in the relative proportion of the individual groups of organisms which comprise this flora. This suggested that the normal bacterial flora is acclimatized to the intestinal environmental conditions of temperature, reaction and composition of food, and of intestinal secretion at different levels. It also indicates that the activities of the organisms which comprise the normal intestinal flora are not in active opposition to those of the host."

Function of Intestinal Bacteria.—The question has often been raised: are the intestinal microörganisms essential for the satisfactory digestion of food, or should they be regarded as true parasites? Pasteur believed that they are essential. "I do not conceal the fact that had I time to undertake this study I should do so with the preconceived opinion that life under such conditions would become impossible, (*i.e.*, without intestinal bacteria). Given that such experiments are capable of being gradually simplified, it might be possible to study digestion by adding systematically to the sterile food of which I speak various individual bacteria or different bacteria together, each of definite known species.

"The hen's egg lends itself without serious difficulties to experiments of this kind. Previously freed externally from every

sort of living impurity just before hatching, the chick should be immediately put into a chamber free from every sort of bacterium so that it could be supplied with pure air and sterile food easily introduced from without in the shape of water, milk, and corn.

"Whether the results were positive, confirming the preconceived idea which I am putting forward, or negative, or even absolutely opposite, *i.e.*, life without bacteria is easier and more vigorous, in any case the experiment would be full of interest."

Several investigators have attempted to conduct experiments in the manner suggested by Pasteur. The conclusions are uniform in one respect—that the experiments are beset with far greater difficulties than Pasteur anticipated. Nuttall and Thierfelder tried the following experiment: Guinea pigs were removed shortly before birth from their mothers by cesarean sections. Suitable precautions were taken so that they did not become infected. They were then placed into sterilized cages, given sterile food, water, and air. Such animals gained in weight normally. Later, experiments of the type Pasteur had suggested were conducted on chickens. The surface of eggs was carefully freed of bacteria, incubated and hatched in germ-free incubators. The resulting chicks were kept in a germ-free environment, given sterile food, water, and air. These chicks ate well but did not make proper growth. Similar experiments were conducted on turtles and on frogs. The results in many cases were inconclusive. Probably in a large measure the inconclusive results may have come from the feeding of inadequate diets. As it is conceivable of a diet being sufficiently balanced when fed to animals with an intestinal microflora and insufficient when fed to animals with a sterile alimentary canal, the results of Lewin are more convincing. He found the intestines of a number of Arctic animals sterile, yet they functioned normally.

Loeb and Northrop's experiments on the fruit fly shed considerable light on two questions: (1) Are intestinal bacteria essential for normal life? (2) Do bacteria growing in the alimentary canal elaborate poisons which injure the body of the host? Freshly laid fly eggs were placed for a few minutes into a bichloride of mercury solution of sufficient strength to kill all the microörganisms on the surface. Only a few of the eggs thus treated survived. These were kept in sterile flasks on sterile meat and developed into normal flies. These were carried through 87 aseptic successive generations on sterile yeast. At the close of the experiments their bodies were analyzed and found to be sterile

inside and out. They were apparently normal in every respect except that they were free from microörganisms. Their average span of life was 28.5 days. Pearl found the average life span of non-sterile flies grown at the same temperature to be 31 days. Similar results have been obtained by others. Consequently, the conclusion has been reached that natural death is not, as suggested by Metchnikoff, due to poisons elaborated in the intestines by bacteria, but rather that old age and natural death are caused either by the gradual production in the body of sufficient quantities of harmful or toxic substances, or by the gradual destruction of substances required by the body to keep it in youthful vigor. Under natural conditions both factors may contribute their part in causing old age and death. Consequently, it may be concluded that intestinal bacteria are not essential to digestion and normal health. Still, it is evident that they do play a beneficial, if not vital, rôle in life. Naturally some harmless and some injurious bacteria will reach the alimentary canal. Now, it is well-known that the products of some bacteria, particularly those which produce acids, will inhibit the growth of others; consequently, intestines seeded with harmless parasites are protected against injurious ones just as well-seeded meadow is protected against weeds.

Injurious Action.—It is quite a general experience that constipation gives rise to certain symptoms which consist in the main of mental haziness, "dopiness," malaise, headache, coated tongue, poor appetite, and so-called "biliousness." These have been ascribed by different individuals to various causes. Johann Kampf taught that all diseases are due to fecal impaction, and this idea was held by many writers of the eighteenth and nineteenth centuries. Its greatest champion was the Russian bacteriologist, Metchnikoff. He and his followers maintained that many bacteria grow in the intestines and elaborate certain toxic compounds: hydrogen sulfide, indol, skatol, phenols, cresols, histamins, and others which are absorbed from the bowels, circulated in the blood, and which cause the symptoms. Moreover, it was claimed that the constant entrance of these products into the blood gave rise to nervousness, hardening of the arteries, and changes in the kidneys and other vital organs. Metchnikoff and co-workers looked upon the large intestine as a useless and injurious organ which had developed in the evolution of man. Lane attempted to remedy this defect by spectacular surgical procedure, but this was accompanied by such a tremendous mortality that at the present time it is resorted to only under

necessity, *i.e.*, in diseased conditions of the colon. Metchnikoff's observations led him to advocate a different procedure. He knew that lactic acid has a preserving effect, and as we have learned, is often used even today in the preservation of certain foods. Furthermore, it was known that the Bulgarians appear to enjoy a somewhat unusually long span of life. They consume large quantities of sour milk; hence, the conclusion was that drinking sour milk prevents putrefactive changes in the intestines which if permitted to occur would result in illness and premature death. Thus, it became quite a common practice to prescribe sour milk with the belief that by so doing the natural intestinal microflora was being supplanted by that of the *Lactobacillus bulgaricus*. Later bacteriological work has shown that although this organism grows readily in milk, it does not become established in the intestines. This has led to the use of other bacteria especially *Lactobacillus acidophilus*.

Alvarez maintains that the symptoms associated with constipation and referred to as autointoxication may in the child be caused by the great absorption of toxic products from the intestines, but this cannot explain the whole condition in the adult for the following reasons: (1) A great amount of work has been done in an attempt to isolate substances which are produced by intestinal bacteria and which will produce the symptoms associated with autointoxication. Investigators have reported the finding of such products, but others have failed to confirm these findings. Hence, it would be wrong to state that bacteria produce no such toxic products in the intestines, but it can be stated that the search has failed to reveal such a product. (2) Increased blood pressure, which is believed by some to be caused by autointoxication, is met with more commonly among boys than among girls, yet constipation is more prevalent among the latter. (3) In constipation the fecal mass is dried out; hence, bacterial activity and absorption of toxic products would be less than in diarrhea. (4) The lower part of the bowels absorbs from the blood and does not excrete into the blood, consequently, bacterial products to be injurious must be produced and absorbed before the material reaches the colon. (5) Symptoms similar to those associated with constipation have been produced by packing the lower bowel with inert substances. These cannot be absorbed but give rise to an antiperistaltic action with the resulting symptoms of constipation. (6) Relief comes almost instantly on emptying the bowels, a condition which would not be

expected if poisonous products had been absorbed, for it would then require time for their removal from the body. Even Alvarez agrees that it is very probable that at times there may take place a production and an absorption of harmful toxic products from the digestive tracts, but this is much rarer than the literature on the subject would lead one to believe. As in the past, the term autointoxication has been used to include all poorly understood diseases for which there is little or no evidence of their being due to intestinal-produced poisons.

The diet has a marked effect upon the intestinal microflora and regulates an individual's sense of well-being. This is vividly shown by the experiments on monkeys by Herter and Kendall. It is known that eggs and meat encourage the growth of putrefactive bacteria in the colon, whereas milk and glucose stimulate the growth of fermentative lactic-acid forming organisms. Monkeys were placed first on a diet which would favor putrefaction, and then later changed to one which would favor fermentative changes. When placed on the meat and egg diet the animals became sleepy and rested their heads upon their hands in a bowed position. They were stupid and responded slowly to external stimuli. They took their food very deliberately and manifested little interest in their surroundings. Not infrequently after a hearty meal they would spend much time trying to bite the woodwork of the cage. The urine was of small volume and amounted approximately to one-half that produced on a milk-carbohydrate diet. The amount of putrefactive products in the urine was increased. When the animals were changed to the milk-glucose diet, both the physical and psychical attitudes underwent a great change. They no longer held their heads in their hands but assumed an upright position. Their appetites were keen, their eyes became bright, they showed more interest in their surroundings, and the putrefactive products in the urine decreased.

Influence of Putrefaction on Infection.—Metchnikoff believed that some microörganisms may assist others in entering the body from the intestines. He made streak inoculations on gelatin plates with cholera organisms. Crosswise of these he inoculated certain putrefiers. At the points where the lines crossed larger colonies of the cholera germ were formed than elsewhere on the plates. Repeating this experiment with acid producers there was no growth of the cholera organisms at the points where the lines crossed. Consequently, he concluded that putrefiers accelerate

the growth of cholera germs, whereas acid producers inhibit. He next conducted experiments on animals. Guinea pigs, rabbits, cats, and other animals are refractive to cholera, but Metchnikoff found on feeding young rabbits putrefiers and then the cholera germ, that these animals developed a disease similar to cholera. Furthermore, Meyer has found that guinea pigs, which are normally immune to the typhoid bacilli that enter through their mouths, become infected if they receive the germs when they are on a diet of bread and milk.

It is general information that the intestines of the child are more permeable to bacteria than are those of the adult, and it is maintained by Park that much of the injury resulting from the feeding of poor grades of milk during the summer months comes from certain bacteria which are normally nonpathogenic permeating the intestines. This may result from lowered vitality brought about by heat, associated with microörganisms, or possibly there may be certain vitamin deficiencies. Nevertheless, it opens up a new and interesting lead as to the cause of certain heretofore ill-understood disorders which in the past have been attributed to autointoxication. Hence, Adami and others feel that it is much more rational to regard the ill effects of intestinal stasis in man as caused not by autointoxication, but by conditions favoring subinfection.

How to Modify Intestinal Microflora.—Numerous attempts have been made to decrease or to change the intestinal microflora. Sterile food has been fed and found to have little effect in reducing the number of microörganisms of the intestines. Complete starvation has often resulted in an increase, and many intestinal antiseptics have been tried (and some are still being used), but practically all of those workers who have taken the trouble to make bacterial counts before and after the administration of these drugs admit that they have little or no effect.

Numerous investigators have attempted to control intestinal putrefaction by feeding acid-forming bacteria together with carbohydrates. Escherich used *B. lactis aërogenes.* Quicke suggested the use of yeast, *Oïdium lactis,* and the writings of Metchnikoff gave this method a great impetus. He considered the failures of others as due to the use of organisms having weak acid-producing powers; consequently, he advocated the use of *Lactobacillus bulgaricus* which was isolated from milk and has been proved to produce as much as 25 grams of lactic acid per liter of milk. He advocated the use of the organism either in milk or

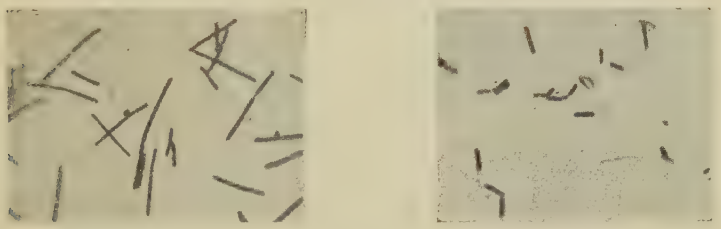
broth cultures. His enthusiasm, together with the simplicity of the process, has almost made a fad of it in some localities and with some persons it is considered a cure-all. Yet, the mere feeding of *Lactobacillus bulgaricus* for the purpose of antagonizing putrefactive processes in the intestines has been disappointing. It has been found that although the organism grows readily and produces large quantities of acid in the test tube, it fails to become established in the intestine.

The use of *Lactobacillus acidophilus*, which is a normal inhabitant of the digestive tract, seems to be much more promising. Extensive work has shown that the intestinal microflora can be changed not only by giving massive cultures of *Acidophilus bacilli* but also by giving large quantities of lactose. The lactose is so poorly absorbed from the bowels that it gets down into the lower ileum and colon and there modifies the culture medium to such an extent that the sugar-fermenting bacteria can outgrow the purefactive ones.

Kopeloff has been able to change quite permanently the microflora of the intestine by feeding milk containing massive doses of *L. acidophilus*. He used 1,000 c.c. of a culture containing 200,000,000 viable *L. acidophilus* organisms per cubic centimeter. By this means he was able to overcome bad cases of constipation and to prove that the effect was bacteriological and not physical, or chemical. He showed: (1) That patients given large quantities of sterile milk were not relieved of constipation; (2) that the feeding of large quantities of milk containing the dead bodies of *L. acidophilus* and their products failed to relieve constipation; and (3) that *L. acidophilus* milk from which the organisms had been separated by filtration also failed to cure constipation. But, when milk was fed containing massive doses of the living *L. acidophilus* organism, the results were always favorable. Most authorities are agreed that the tablets and other preparations on the market are practically valueless, as may be seen from the following statement of Bass:

"In the instance of tablets, none of these examined was found to contain as much as 1,000 viable bacteria of any kind per tablet. If it should be granted that all the viable bacteria present were *B. acidophilus*, it would take about 1,000,000,000 tablets, or more than 20 tons, to contain as many bacilli as are contained in 1,000 cc., or the usual daily dose of the acidophilus milk, the quantity found, by most investigators at least, to be necessary to transform the flora."

Similar results were obtained in liquid cultures which are often attractively labeled and tastefully flavored but contain so few bacteria that a patient would have to drink about seven to eight gallons daily to get the required dose. Consequently, where the organisms are to be used, the starters must be obtained from reliable laboratories and grown in milk under appropriate conditions. Encouraging as have been the results in modifying the intestinal microflora by *L. acidophilus*, the most effective and



a b

FIG. 90.—(a) *L. bulgaricus*, (b) *L. acidophilus*. (*After Kulp and Rettger.*)

a b

FIG. 91.—(a) Agar colonies of *L. bulgaricus*, (b) Agar colonies of *L. acidophilus*. (*After Kulp and Rettger.*)

most permanent method is still the use of a suitable diet, particularly one containing large quantities of lactose and maltose.

As regards the *L. acidophilus* it might be well to add that it is a gram-positive bacillus which easily undergoes granular degeneration. It grows under acid conditions not tolerated by most other bacteria. It is a non-motile rod, 1.5 to 2 microns long and 0.6 to 0.9 micron wide with slightly tapering ends. The optimum temperature for growth is 37° C. In milk, growth occurs slowly, taking about three weeks for complete curdling. It produces no gas and does not form spores. It is non-pathogenic for animals.

CHAPTER XXX

BACTERIA AS THE CAUSE OF DISEASE

Bacteria govern the length of man's life and determine the part of the earth on which he may live. They have defeated armies and destroyed cities. Nations which have been the glory of the world have gone down before them. The majority of individuals born today would enjoy health and live to old age were their bodies not invaded by microbes. They are no respector of persons, nor are they governed by the edicts of rulers. However, at the command of modern science they are commencing to relinquish their hold, and some of earth's most fertile regions, where in the past the risk of death to the visitor was greater than a visit to a battlefield, are being transformed into tropical health resorts. Some microbes are no longer the fairy tale adventurers of death and disease. They are being controlled; disease is being reduced; and during the past century man's expectancy of life has been doubled. Still much remains to be done for the learning and the practicing of the laws of sanitation can prevent more suffering and save more lives than the abolition of war. Here the scriptural entreaty is especially appropriate: "Give me understanding and I shall keep the law; yea, I shall observe it with my whole heart." Each bit of knowledge gained in this field carries man one step nearer the goal where he will be the master and not the slave of his environment.

Theories of Disease.—The methods used in the prevention and cure of disease are determined by man's ideas concerning the nature and cause of disease. Man's earliest conception of disease was that it was due to demons. These were supposed to enter the body of their victim and rule in a merciless fashion. "The possessed man tossed and shaken in fever, pained and wrenched as though some live creature were tearing or twisting him within, rationally finds a personal spiritual cause for his sufferings. In hideous dreams he may even see the very ghost or night-mare fiend that plagues him. This is the savage theory of demoniac possession, which has been for ages, and still remains,

the dominant theory of disease and inspiration among the lower races. It is obviously based upon an animistic interpretation, most genuine and rational in its proper place in man's intellectual history, of the actual symptoms of the case."

Such was the belief concerning disease amongst the early ancestors of modern civilized man, and such is the belief of primitive man even today. Logically, such a disease could be cured only by dislodging the invading demon, and what was more natural than to conclude that this could be accomplished by either force or by flattery? The medicine man attempted to force out the invader by making the habitation unbearable. The victims were whipped, loaded with chains, given ill-smelling and bad tasting drugs, all in the hope that the demon would seek more congenial quarters. Often attempts were made to entice him out. Here charms, incantations, and sacrifices played a prominent part. For centuries roots and herbs were prescribed because of their symbolic form. They used curious things such as the moss scraped from the head of a culprit hanged in chains, or from mummies from the Egyptian tombs, or the roots of plants gathered in a graveyard during the dark of the moon. It was not until man discarded the belief that insanity is a manifestation of demonic possession that the last vestige of the demonic theory disappeared from the medical idea of civilized man.

Hippocrates, who is known as the father of medicine and who lived during the Middle Ages, was one of the first to teach that disease is due to a derangement in the body itself. He taught that the body contains four humors: blood, phlegm, yellow bile, and black bile. When these were present in the right proportion, one enjoyed good health. Small variations accounted for differences in temperament; a sanguine temperament was due to a preponderance of blood; a phlegmatic to an excess of phlegm, and a bilious, or melancholic temperament to a surplus of bile. Greater variations resulted in illness; hence, it was the object of the medical profession to keep the proper balance of the humors and in this way to maintain health.

Hippocrates' theories were not seriously questioned until the seventeenth century when a flood of new theories burst forth. Some of these were even more complex and mystical than the ones they were intended to supplant. Disease was regarded as "an intestinal movement of particles," "a struggle between nature and disease-producing matter," "a lack of tone or an insufficiency of tone." There was the dynamic organic system

of Stahl, who believed that the soul was the supreme principle of disease. There was the mechanico-dynamic system of Hoffmann, which stated that life expresses itself in motion, and that all manifestations in the body are controlled by nervous spirits. The school of Montpelier taught that various organs possess individual life. Mesmer claimed that magnetic fluids flowed from the hand, and the Bremontau system asserted that all that was necessary for a cure was to determine the grade of a disease in accordance with the strength or weakness of the active irritation, and to adjust the right proportion of strengthening or weakening medicine to the case. The theory which lasted longest and had the greatest effect upon medical practice was the theory of homeopathy founded by Hahnemann (1775–1843). He defined disease as a "spiritual dynamic derangement of a spiritual vital principle." His theory was that the potency of drugs was increased by dilution provided the dilution was accompanied by shaking. In some cases it was also supposed to be increased by pounding. Accordingly, he and his followers diluted their drugs to an enormous extent. They also tested them upon themselves. In the case of ordinary tinctures he recommended diluting two drops with 98 drops of alcohol, taking two drops of this mixture and adding 98 drops of alcohol, and repeating the process 30 times. Virchow commented on the folly of this doctrine by saying that he would defy even a German to detect beer at the mouth of the River Spree after a glass of it had been spilled into the river seven miles upstream. Hahnemann taught that in curing disease, a knowledge of anatomy and physiology was unnecessary, and that pathology was a hindrance. Not only was the dosages he recommended always extremely small, but many of his preparations were of a highly poetic and romantic nature. There were: *lachryma filia*, the tears of a young girl in great grief and suffering; *flavus irides*, the yellow rays of the spectrum; the extract of three kinds of lice, and the like. It is almost incredible that such a doctrine could exist, not to say flourish, for nearly half of the most progressive of all the centuries. Yet it did, and was in the end actually an advance over some of the previous methods that had been emphasized in which prescriptions contained upward of one hundred ingredients. Some of the old prescriptions had been built up by succeeding generations of doctors, each doctor believing that he must add one or more drugs as his contribution to the prescription, or consider his life wasted. Thus, it is not surprising to learn that homeop-

athy cured more than the systems it was replacing. It appeared to substantiate the statement of Oliver Wendell Holmes: "If all the drugs that had ever been used for the cure of human ills were gathered together and thrown into the sea it would be ever so much better for humanity and ever so much worse for the fishes."

"This maelstrom of confused theory was finally directed into a definite current. Louis Pasteur rose like a Triton. By the brilliance of his genius, by the clearness and breadth of his vision, he formulated the bacterial or germ theory of infectious diseases which must forever dominate medicine. No longer could malevolent spirits be held responsible for disease, nor could an improper admixture of the four humors be regarded as the cause of ill health. Disease was but another example of the struggle for existence. It was life preying on life; the invasion of the macroörganism by the microörganisms." The cause of typhoid, cholera, diphtheria, tuberculosis, meningitis, and many other diseases have today passed from the realm of theory into the field of established fact, and each year finds the list of vague indefinite diseases growing shorter and the list of germ diseases longer.

Pathogenicity.—Continually surrounded as we are by bacteria, the mystery which confronts the beginner in bacteriology is not why men die of microbial infection, but really how it is possible for any of them to escape death for so long a period. The student, however, soon learns that this is due to a number of reasons. He soon learns that microörganisms are of three classes: saprophytes, parasites, and pathogens. Saprophytes live on dead organic matter and transform it into such forms that the higher plants and animals can use it again. Saprophytes are the most numerous and most important forms of microscopic life. They rarely initiate a disease in man or the lower animals, but are occasionally found associated with diseased conditions as secondary invaders. They usually gain their foothold and do their work after other microörganisms or conditions have broken down the natural barriers. These bacteria comprise that great group of microörganisms chief among which are fermenting, putrefying, and decaying bacteria that play such a prominent rôle in the cycles of the elements. They are widely distributed in nature, and many of them form resistant spores which carry them over long adverse periods.

A smaller group of microörganisms is made up of parasites which exist upon, or even in the body of the living plants and

animals. They invade and give rise to disease only when the natural barriers are broken down. They are seldom found far from the bodies of their hosts. The colon bacillus is an example of this group. Its natural habitat is the gastro-intestinal tract of man and many animals. Under appropriate conditions it may invade the body of its host and give rise to appendicitis, cystitis, peritonitis, and other inflammatory processes. Bacteria often add the finishing touch in many diseases. To this same group belong the streptococcus, the "blood poison organism;" the staphylococcus, which causes boils and carbuncles; and the various bacteria which exist in the upper part of the respiratory tract, and under favorable conditions give rise to pneumonia. Parasites have very appropriately been designated by Theobald Smith as "opportunists." They seldom initiate a disease, but are often present and enter the body as secondary or terminal invaders. They are usually found only in association with the bodies of plants and animals, and are usually comparatively frail. A few, like the bacilli of botulism, lockjaw, anthrax, and the gas bacillus form spores and survive for long periods under conditions which quickly kill the true pathogens.

A still smaller group is that of the pathogens, microörganisms which attack man and the lower animals. Some of these organisms are pathogenic only for man, others only for the lower animals. A few can cause disease in both man and the lower animals. The true pathogens with few exceptions can multiply only in the body of their host, and pass rather quickly from the body of one animal to that of another. Probably they have evolved from the parasites, which in turn had their origin as saprophytes. The sequence of events probably was: saprophytes, parasites, pathogens. It is quite probable that the saprophytic bacteria were the first to render the surface of the earth fit for higher plants, which in turn furnished food for animals. These same saprophytes may then have accidentally reached the surface of the bodies of living plants and animals, where most of them were dislodged or died; while a few adapted themselves to their new environment and became parasites. Eventually some of the latter may have invaded the living tissue, and became pathogens. Such a theory raises the probability of new parasites and new pathogens being evolved even at the present time.

The nature and extent of the disease produced by a pathogen according to Kendall depend upon the following factors: (1) The kind of microörganism; (2) the number of microörganisms;

(3) their ability to locate and force an entrance into the tissues of the body; (4) the location and the extent of their multiplication in the tissues of the host; (5) the reaction of the tissues of the host to the invader; and (6) the nature and extent of the secondary specific defenses of the host against the invader.

The power of invasion is not fixed. In many cases it may be increased or decreased. Pasteur increased the invasive power, virulence, of the virus of rabies taken from dogs for rabbits by passing it successively through the body of different rabbits. Usually when passed through the bodies of specific animals bacteria increase in virulence for that species of animal, but decrease for others. Pasteur also found that the microörganism which causes chicken cholera when grown on laboratory media, as well as the anthrax bacilli grown at an abnormally high temperature decrease in virulence. Organisms which are reduced in virulence by any means are spoken of as attenuated. Attenuated organisms are often used in rendering animals immune to disease.

How Pathogens Reach the Body of Man.—Most pathogens multiply only in the body of man and the lower animals; hence, they must leave the body of the diseased and reach the body of a healthy animal before they can initiate a disease. Before they enter the body of man, they must pass a few mechanical barriers and a few chemical bulwarks, which constantly stand between man and the invading hordes. Some bacteria, such as diphtheria and tetanus, grow on the body and elaborate a poison which is absorbed by the tissues and gives rise to the specific disease. Others, like typhoid and anthrax, multiply in the body tissues. It is, thus, quite evident that the ease with which the pathogens are distributed depends largely upon the nature of the disease which they produce. When, as is often the case with the tubercle bacilli, they enter the bones they cannot readily leave the body of the host, and secondary cases do not occur. When pathogens, such as the typhoid bacilli, enter the blood stream and leave in the urine and alvine discharges, the liklihood of their reaching a second individual is less than when they leave in the secretion of the nose and throat, as in the case of diphtheria, scarlet fever, and influenza.

Having left the body of their first host the pathogens must find a second or perish. Most of them perish, but a few reach susceptible individuals to whom they are conveyed by direct contact—the fingers, objects which have been mouthed by the diseased, various insects, and food. Inanimate objects which are

spoken of as fomites may convey the more resistant, but it is becoming more generally believed that this method of conveyance is of secondary importance, as compared with contact infection where the fresh infected secretions of the diseased are rather quickly conveyed to a second individual. It was seen in an earlier chapter that droplet infection may be conveyed for a few feet, but true air-borne infection plays little if any part in the spread of disease. The manner in which the virus of the communicable diseases are transferred from individual to individual is spoken of as the route of infection. The portals by which microörganisms enter the body are referred to as the channels of infection, and may be grouped under three headings: (1) The skin; (2) the respiratory tract; and (3) the digestive tract. Some organisms may enter by two or even three channels, but many usually enter by a specific one.

Park illustrates this as follows: If very virulent streptococci, typhoid bacilli, and diphtheria bacilli are rubbed into an abrasion of the skin, the typhoid bacilli produce no lesions, the diphtheria only a very slight area, but the streptococci may give rise to a severe or even a fatal septicemia. If the same organisms are placed in an abrasion of the throat, the typhoid is again harmless. The diphtheria organism may give rise to diphtheria, and the streptococci, tonsillitis, or even septicemia. Finally, if the three organisms reach the intestines, it is the typhoid which causes a diseased condition, and as a rule not the others. If the tetanus organism reaches an appropriate wound on a man or horse it gives rise to that highly fatal affliction, lockjaw. Yet the normal habitat of this pathogen is the intestinal tract of the horse, and often the alimentary canal of man.

According to Rosenau approximately 90 per cent of all infections enter the body through the mouth with the various objects which are placed in it: fingers, eating and drinking utensils, food, water, and so on. This is probably one reason why children have more communicable diseases than adults. It also makes this the most important channel to guard in preventing the spread of communicable diseases.

Mechanical Barriers.—After microörganisms reach the surface of the body they must pass certain mechanical barriers before they enter the body and can cause a pathological condition. The outer defenses are the skin and mucous membrane. The skin is constantly covered with various microörganisms, most of which are transient saprophytes that come and go irregularly.

A few, such as the pyogenic cocci, occur with such regularity as to be considered provisionally as habitual parasites. To these hordes which hover about us, the skin presents an almost impenetrable barrier. Some cocci and the plague bacilli when vigorously rubbed into the sweat glands and hair follicles may penetrate the underlying tissue and cause disease; but even with these, this is exceptional for they usually enter through some cut or abrasion, a severe wound, the slight lesion of a hang nail, or the tiny bite of an insect. In addition to the pyogenic cocci the organisms which cause tetanus, anthrax, symptomatic anthrax, malignant edema, and the gas bacillus occasionally enter the damaged skin and cause either localized infection or widely distributed injury.

Because of their moisture and warmth, the mucous membranes covering the inner surfaces of the body occasionally invite bacterial multiplication. In the case of the nose and upper respiratory passages, the moist mucous holds the bacteria, thus preventing their entering into the deeper alveola of the lungs. Even here the healthy mucous membrane is a nearly perfect barrier, for most individuals harbor pathogens in the upper respiratory passages which if permitted to enter the tissue might give rise to disease. The lung is often damaged by irritating fumes, sharp bits of straw, rock, emery or glass, and pneumonia quickly results. During health the constant rhythmic contraction of the cilia carry upward and outward the bacteria which may have penetrated the bronchi and bronchioles.

The eyes are presided over by the faithful eyelids and eyelashes which keep many would-be intruders at bay. The polished surface and the constant flow of tears carry most invaders out and under ordinary conditions prevent infection. When the tear ducts fail to function normally as, for example, when the individual does not receive sufficient vitamin A in his diet, infection becomes more prevalent. Normally some bacteria continually find their way to the eye, and the *B. xerosis* occurs with such regularity in the conjunctival sac as to be regarded as a normal inhabitant. The gonorrheal infection often enters by this channel, and in the case of the child is probably the greatest single cause of blindness.

The mouth is a veritable garden of microörganisms, saprophytes, parasites, and often pathogens; some of the latter occasionally invading the salivary glands. These organisms find a suitable home in every crevice and region of the mouth.

They cause dental caries and often penetrate to the very root of the teeth and cause not only loss of the teeth but often constitutional diseases. The normal secretion of the saliva washes many from the surface of the teeth and keeps them on the move. When for any reason normal secretion of the saliva fails, the teeth and gums quickly become coated with bacterial films which give rise to ill-smelling and tasting products. Moreover, individuals who constantly sleep on one side usually lose the teeth on the opposite side of the mouth earlier, than those on the side on which they sleep. This is probably due more to the failure of being constantly washed by the saliva than to its slight bactericidal properties.

The microflora of the mouth are constantly being washed into the stomach where they come in contact with the gastric juice, which is highly unfavorable to their life and multiplication. This is due to the hydrochloric acid which it contains. But even this partakes more of the nature of an antiseptic than a true disinfectant. It has been established by experiments that the typhoid, tuberculosis, and dysentery bacteria rather readily pass the stomach when swallowed with food. Water and many liquid foods quickly leave the stomach with a low acidity. Moreover, bacteria may be imbedded in large particles and thereby be protected from the hydrochloric acid. Nevertheless, the gastric juice plays a prominent rôle in the destruction of saprophytes and pathogens, which in its absence would survive and reach the highly favorable medium in the intestines and quickly multiply there. That hydrochloric acid protects against disease is certain from the work of Koch, who found it impossible to infect some animals with certain bacteria until he had neutralized the gastric hydrochloric acid. The gastric juice destroys tetanus and diphtheria toxins but is without effect upon the botulism toxin. Investigations show that the stomach is exceptionally free from microbial infection.

Most bacteria which reach the intestines find the partly digested alkaline mass highly favorable; hence, they rapidly multiply, and by the time the mass reaches the descending colon each gram contains billions of microörganisms.

Considerable antiseptic action was formerly attributed to the bile, but that this cannot be great is indicated by the fact that typhoid bacilli have been found growing in the bile ducts and gall bladder. It appears to favor the growth of the colon typhoid group of bacteria. Bile increases peristaltic action and serves

as a slight laxative, thus giving less time and opportunity for the pathogens to enter the body. Parasitic bacteria like the streptococci and *Bact. coli* often occur in the intestines in large numbers, but normally do not permeate the intestines. However, when the body resistance is decreased, these may invade and cause disease. Some authorities consider that hot weather reduces body immunity in the case of small children, and that digestive disturbances in the summer months are more often due to the entrance of parasites than to true pathogens. Even pathogenic bacteria, such as the typhoid, dysentery, and tubercle bacilli, may pass through the whole length of the intestines without causing inflammation. Slight lesions aid their passage into the deeper tissue.

Number as Related to Resistance.—The body may successfully cope with a few bacteria but may be overcome by many. Hence, the number of bacteria which enter the body may be the determining factor. The minimum number which must run the gauntlet of the body defenses varies with the microörganism and also with the animal. The statement is often made that "ten tuberculosis bacteria are necessary to inoculate a rabbit." Ordinarily, man can handle a few tubercle bacilli without becoming infected, but massive doses are quite certain to cause infection; some other microörganisms, as for instance the anthrax bacillus, may infect a very susceptible animal when few enter. Probably in most diseases a great number of the specific microbes have to enter before the normal defenses are broken down.

How Bacteria Produce Disease.—When bacteria or their products enter the body they cause various changes within it. The three most important effects are: (1) destruction of body cells or tissue; (2) the formation of toxins; and (3) the formation of poisonous "split-proteins." The staphylococci and streptococci destroy body tissue in boils and abscesses, and in septicemia they destroy the red corpuscles. The typhoid, cholera, and dysentery bacilli destroy patches in the intestines. The pneumonia and tuberculosis bacteria destroy the tissue of the lungs, and at times give rise to fatal hemorrhages.

The diphtheria and tetanus organisms multiply at the point of entry and elaborate a toxin which is absorbed into the system and causes the injury. The majority of bacteria on entering the body are broken down with the liberation of toxic substances, known as "split-proteins." The main differences between the true toxin and toxic products, "split-proteins," are thus summar-

ized by Broadhurst: "(1) True toxins are formed by living cells; poisonous 'split-proteins' only on the death and disintegration of the bacteria or of similar non-living proteins. (2) True toxins are produced by only a few species of bacteria, but the poisonous 'split-proteins' may be obtained on the decomposition of any kind of bacteria, or, apparently, any protein substance. (3) True toxins, being soluble and therefore extra-cellular, are found in the filtrate of the bacterial cultures, while the poisonous 'split-proteins' are liberated only on the destruction of the cells themselves. (4) Toxins stimulate the animal body to produce anti-toxins, but the poisonous 'split-proteins,' on the contrary, do not excite such anti-body reactions. The most that is claimed for the 'split-proteins' is a kind of tolerance, not a definite immunity."

CHAPTER XXXI

IMMUNITY

Bacteria enter the body by one channel or another. They get into the lymph, or the blood stream, and finally into the tissues. At times they gain entrance directly into the tissue as a result of a wound. They have passed the first line of defense, but here they meet other defenses. These are not mere passive obstructions, as there results an actual biological struggle between the host and the invader. Usually the conflict is a quick, decisive one, and the invader is destroyed. Often the microbe establishes a foothold, multiplies, and carries on an offensive warfare, in which case the tissues of the host mobilize their defensives, the weapons used depending upon the specific invader. In most instances a real chemical warfare ensues which ends only in the death of the invader or the invaded. There can be no armistice. On being attacked, some germs become intrenched. Then follows a waiting fight; the host constantly throws up barriers, while the invader ever ready to take advantage of any weakening in the defense, immediately begins to wage an aggressive campaign when the weakening comes. This may end in a secondary intrenchment, the complete routing of the invader, or the death of the invaded. This eternal warfare is inherent in all animals, and the science which deals with the process is known as immunology.

Immunity is Universal.—If one examines a drop of stagnant water under the microscope it is found to contain minute forms of animal life, ameba, and minute forms of plant life, bacteria. On careful examination the ameba are seen ingesting and digesting the bacteria. Now, if one kills the ameba and again examines them the living bacteria are observed decomposing the dead ameba. It is evident that there was something inherent within the living naked mass of amebic protoplasm which protected them against the bacteria. If a living plant be carefully examined microscopically its surfaces are found covered with myriads of microörganisms, yet the living plant is uninjured by them. If this same plant is killed and left in a moist place,

the same bacteria quickly tear it to pieces. On the skin, in the upper respiratory passages, and in the alimentary canal of man and the lower animals are millions of different microörganisms, yet during life they do not attack. Something happens—we call it death—and the microörganisms quickly enter all the tissues. If it is a friend who is thus attacked and we are far distant, we have to hurry to obtain a last fond look before the wrecking crew tears the body to pieces.

Then too, the germs which attack man during life are often not the ones which attack the lower animals. Animals can drink with impunity water infected with typhoid and cholera, yet man, on drinking similar water, becomes infected. Fowls do not sicken with Asiatic cholera, nor men with fowl cholera. Whooping cough and diphtheria are unknown among the lower animals, and hog cholera and Texas fever are not among the human diseases. This power of resisting infection in varying degrees is universal and is known as immunity. As may be seen from the above examples, this power is not absolute and varies with different plants and animals and under different conditions, but it is possessed by all life in some degree; for the absence of immunity, like the absence of bacteria, is incompatible with life on this planet.

Natural Immunity.—If one glances over the list of diseases to which various species and races of animals are victims, it is apparent that some animals are never naturally infected with many of the microörganisms that cause extensive and fatal ravages in others. The lower animals are immune to gonococcus, diphtheria, typhoid, measles, scarlet fever, and other diseases; whereas man is immune to chicken cholera, canine distemper, hog cholera, and others. This is due to natural immunity, which may be roughly defined as the immunity certain individuals possess by virtue of their belonging to a given animal species. It is inherent to a greater or less extent in all members of the species. In some cases, at least, it is due to the microörganism failing to find suitable cultural conditions in the body of the host. For example, those germs which cause disease in the warm-blooded animals usually are without effect on the cold-blooded, and vice versa. Anthrax does not attack turtles and frogs, nor avian tuberculosis man. It has, in some instances, been found possible to infect an animal with specific microörganisms to which it is ordinarily immune by changing its body temperature. Some diseases of the herbivora do not occur spontaneously in the carnivora.

Dogs, for instance, are relatively immune to anthrax and tuberculosis.

The degree of immunity varies. In some cases it is absolute. The lower animals are immune to typhoid. Man has a fair degree of immunity to the respiratory diseases, dependent upon physical conditions. Susceptibility to these communicable diseases is greatly increased by fatigue, over-exertion, worry, undue exposure to cold or heat, poorly ventilated rooms, alcohol, diseases, and insufficient and improper food. Susceptibility to most of the communicable diseases is probably independent of the individual's physical condition.

Just how adverse conditions bring about increased susceptibility to the respiratory diseases is not well understood. Fatigue and overexertion slightly change the alkaline reserve of the blood, which may decrease immunity. Cold and over-heating inactivates certain defensive substances of the blood. Moreover, it has been observed that cold paralyzes, or at least decreases to a marked extent, the movement of the cilia which cover the upper respiratory passages. Consequently, microbes are permitted to penetrate deeper into the more vulnerable terminals.

Variations in susceptibility to diseases have been observed in different races of the same species. This is known as racial immunity. It is claimed that Algerian sheep may be pastured with impunity on land so thoroughly infected with anthrax bacilli as to be deadly for the common breeds of sheep. Field mice are very susceptible to glanders, whereas white mice are immune. The black rat is more resistant to anthrax than the white rat.

Racial immunity is also perceptible in man. It is generally believed that the negro, Eskimo, and American Indian are more susceptible to tuberculosis than the white man. Carroll states that whites are more susceptible to yellow fever than negroes, and that among the latter those living nearest the equator are less susceptible than are the more northern races. Smallpox, which is so highly fatal among some races, is considered a relatively mild disease in Mexico. There seems to be no doubt in some cases that there is an actual racial immunity, but in others the variation may be due to racial differences in sanitary habits.

It is general knowledge that some families or members of a family contract all the communicable diseases with which they come in contact, whereas other individuals of the same family or race escape. When a town's water-supply becomes infected

some individuals contract disease while others escape. This is known as individual immunity. Laboratory animals, rabbits, guinea pigs, etc., on which most experiments are conducted, show very slight individual variation. In fact, the astonishing uniformity of reaction on the part of guinea pigs of similar age and weight against measured quantities of bacterial toxins makes it possible to use these animals to standardize antitoxins. This has led some workers to believe that there is no individual immunity; what is claimed by some as natural individual immunity they believe is really an acquired immunity, and probably due to an earlier slight unrecognized attack of the disease. This factor undoubtedly plays a prominent part in the so-called individual immunity to tuberculosis. Recent work has proved that immunity in the cases of dysentery and typhoid comes from a previous attack. It is quite likely that as the subject of immunity becomes better understood it will be found that the majority of all reported cases of individual immunity to the communicable diseases come from missed mild cases.

There are, however, clear-cut cases of individual immunity due to age. There are certain diseases, commonly classed as children's diseases, to which the individual shows greater immunity at one age than at another: ringworm, diphtheria, and scarlet fever are among these.

Methods of Producing Immunity.—It is quite generally known that one attack of some of the communicable diseases insures the individual against any further attacks. Among the diseases which insure lasting immunity after one attack are the following:

Cholera
Plague
Yellow Fever
Typhus Fever
Poliomyelitis
Smallpox—second attack very rare
Chicken pox—second attack very rare
Mumps—second attack very rare
Scarlet Fever—second attack about 0.7 per cent
Measles—second attack uncommon

In other diseases, such as pneumonia, gonorrhea, and tetanus, there is no lasting immunity regardless of the number of attacks an individual may have previously suffered.

These facts were observed and commented upon by ancient writers, some even suggesting that it might be possible to produce

an artificial immunity. It is stated that Mithridates the Great (132–65 B. C.) attempted to secure immunity against certain poisons by subjecting himself to increasing doses of poisons. His "antidote" contained sixty-four ingredients, some of which were used in infinitesimal amount and "mixed with the blood of Pontic ducks because they lived on poisons." The ancient inhabitants of China and India not only observed this phenomenon, but actually made use of it. They inoculated children with smallpox from active pustules, or had patients sleep in the beds, or wear the shirts of sufferers. These methods were dangerous, but there usually resulted a milder case than those contracted in the ordinary manner.

Artificial immunization first became a success against smallpox due to the work of Jenner. Since that time it has developed along different lines for various diseases. These can all be summarized under four heads:

1. By the Use of Killed Cultures.—This is done in the case of typhoid and some other diseases. The causative organisms are grown on a suitable medium and then killed by heat, antiseptics, etc., after which a known quantity is injected into the body of the individual to be immunized.

2. By the Use of the Toxin.—The organism is grown under suitable conditions, and the toxin separated, after which it is standardized on susceptible animals and usually mixed with an appropriate quantity of antitoxin and then injected into the patient. This is the method used to immunize in the case of diphtheria.

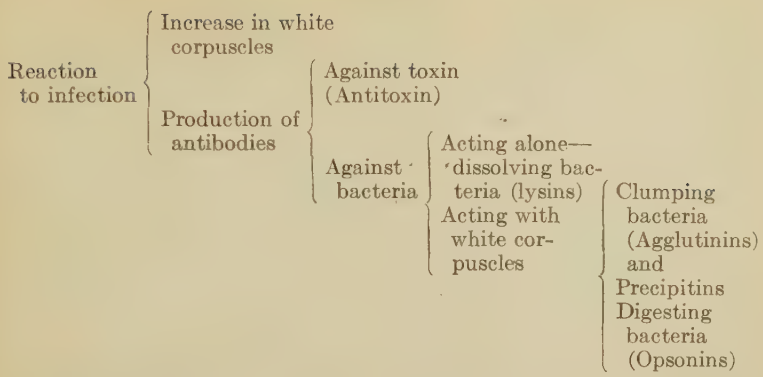
3. By the use of antitoxins which have been produced in the body of a susceptible animal and transferred to the body of the animal to be immunized. This form of immunity is spoken of as passive, whereas each of the other methods are referred to as active.

4. By the Use of Attenuated Cultures.—Attenuation may be accomplished in various ways: (1) The organism is grown in a less favorable animal; (2) it is grown under unfavorable laboratory conditions, such as too high a temperature or in unfavorable nutritive media; or (3) by subjecting the microörganisms after growth to unfavorable conditions, such as drying, chilling, or the addition of some chemical. Such weakened germs are very unlikely to cause serious attacks of their respective diseases, but the fact that there is a possibility, limits to a large extent, their use to veterinary medicine.

Active and Passive Immunity.—Active immunity is always due to the reaction of the individual's own body tissues against microörganisms (living or dead), or their products (toxins or the poisonous products of their bodies). The substances which protect the individual are manufactured in his own body. Passive immunity is always brought about by the introduction of blood or serum from another animal which has previously developed an active immunity. That is, in passive immunity the defensive substances are produced in one animal and then transferred to the one which is to be protected.

Active immunity is a mild attack of the specific disease in the individual who is to be protected; hence, the protection is nearly the same as that which comes from a natural attack of the disease. Immunity develops some time after the microörganism, or its product (the antigen), has been introduced into the body of the individual. There is a reaction on the part of the individual. There usually is slight illness, and certain tissue changes occur during the time the substances, the antibodies, are being developed. The body has learned how to build its own antibodies; hence, the immunity is lasting. In passive immunity the greatest resistance occurs immediately after the serum has been given the individual. There is no negative phase. No illness results from the introduction of the antibodies. The antibodies are foreign substances and are rather quickly disposed of by the body. In passive immunity, the body has not learned to produce its own antibodies; hence, immunity is short-lived.

Reactions of the Body to Bacteria.—The reactions which occur in the body during an invasion by microörganisms vary with different diseases. In some, there is a destruction of the microbes by the white corpuscles; in others, certain soluble substances are produced which inactivate and eventually kill the germs. In still others, substances are formed which neutralize the poisonous products. In most diseases a number of different weapons are used against the invader. Broadhurst tabulates the various reactions as follows:



White Corpuscles.—The white corpuscles are colorless, nucleated cells extremely variable in shape and number. The blood of a normal adult contains from 7000 to 9000 per cubic millimeter. They are of several varieties and may be classified as follows: (1) *Lymphocytes,* which are about the same size as the red corpuscles and contain large spherical nuclei filling almost the entire cell. They comprise about 20 per cent of the white cells. (2) *Mononuclear leukocytes,* comprising from 2 to 4 per cent of the white cells. They resemble the large lymphocytes. (3) *Transitional leukocytes,* which are about the same size as the large lymphocytes with a nucleus that is horse-shoe shaped. They comprise from 2 to 4 per cent of the white cells. (4) *Polymorphonuclear leukocytes,* which comprise about 70 per cent of the white cells. Their nucleus is irregular in shape, usually appearing in three lobulations and sometimes four. Three types of this group are recognized, depending upon their staining.

The white cells protect the body against foreign substances. They possess independent movement; hence, can gather at the site of threatened injury. They can even pass from the blood vessels into the surrounding tissue, where they pick up and digest bacteria or other foreign material with which they come in contact. This activity is called phagocytosis and the cells are known as phagocytes. Most of the ingestion and destruction of bacteria is done by the polymorphonuclear leukocytes. "They are police, judge, jury, and jail all in one. They arrest, engulf, digest, and destroy the criminal germs. If microbic outlaws enter the blood stream, these leukocytes march upon and annihilate them. If the germs enter the tissue the white blood-cells swarm after them, and congregate around the alien horde in

ever-increasing numbers. The felonous host is gradually surrounded, engulfed, and destroyed. The field of combat becomes strewn with the dead and dying corpses of microbes and leukocytes alike. In an ordinary infection, as a boil, the entire mass becomes partly softened and decomposed, necrotic, as the pathologists say, and eventually, forms that gruesome mass known as pus. When the boil is 'ripe'—that is, when the outlaw microbes are fairly under control—the evacuation of the pus usually leads to complete healing. Sometimes the outlaw germs prevail and cause widespread destruction in the body or even death; occasionally, the microbe settles down in a joint, or the heart, and keeps up a languid opposition to the efforts of the body to dislodge them. Usually, however, the leukocytes prevail and restore order."

The phagocytes play a predominating part in protecting us against the pyogenic bacteria; however, recovery from such diseases leaves no immunity as "the leukocytes that are veterans of one insurrection do not ordinarily survive long enough to participate in another. They are short-lived. They leave no descendants."

Injury to the body cells, or infection by bacteria results in inflammation described as follows by Williams:

FIG. 92.

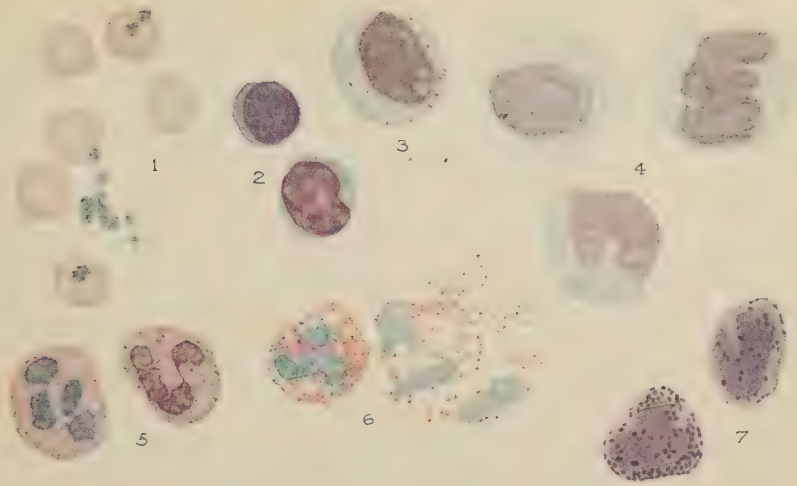
FIG. 1.—The cells of normal blood reproduced from actual cells. Wright's stain (× 1000: 1 mm. = 1μ).

1, Red corpuscles and blood-platelets. 2, Two lymphocytes. 3, A lymphocyte with azurophilic granules. This cell lay in a thin portion of a film and was exceptionally large. 4, Three endothelial leukocytes, one with fine cytoplasmic granules. The granules are rarely so distinct as here shown. 5, Polymorphonuclear neutrophils. 6, Eosinophils, one ruptured. The cells selected for drawing contained fewer granules than are usual. 7, Basophils.

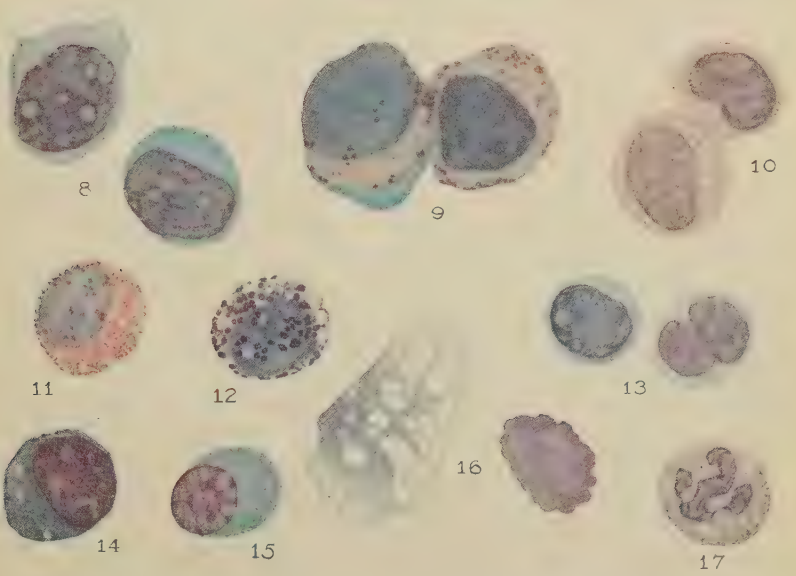
FIG. 2.—Leukocytes found in the blood in disease. All reproduced from actual cells stained with Wright's stain, excepting No. 15, which is copied from Pappenheim (× 1000: 1 mm. = 1 μ).

8, Two myeloblasts, showing nucleoli. 9, Two young myelocytes. Note the blue edge of one. 10, Two mature neutrophilic myelocytes. 11, Eosinophilic myelocyte. 12, Basophilic myelocyte. Some of the granules have dissolved, leaving vacuoles and staining the cytoplasm. 13, Two lymphoblasts, one with lobulated nucleus (Rieder cell). 14, Turck's irritation leukocyte with vacuoles. 15, Plasma cell. 16, Degenerated nuclei, one a so-called "basket cell." 17, Neutrophilic leukocyte with vacuoles.

(*Taken from Todd and Sanford, Clinical Diagnosis.*)



The cells of normal blood (scale: 1 mm. = 1 μ).



Leukocytes which appear in the blood in disease (scale: 1 mm. = 1 μ).

(J. W. Rennell, pinx.)

FIG. 92.—For description, see facing page.

(1) "The first stage is a preliminary contraction of the blood vessels of the part with a later dilation and slowing of the blood-current. The quantity of blood in the part is greatly increased, the small vessels becoming distended.

(2) "In the course of an hour (as observed experimentally in the frog) the blood flow is slowed further, and the leukocytes increasing in great numbers in this area are arranged along the outer part of the blood-stream, the so-called 'margination of the leukocytes.'

(3) "Soon after the margination of the white cells there begins the passage through the walls of the blood vessels. This is the migration of the leukocytes. Reaching the area that has been irritated, these cells engulf irritating particles, or if the active agent is bacteria, they begin battle with these poisonous invaders.

(4) "If the cause of the inflammation is bacterial the migrating leukocytes may completely overwhelm the invading organisms and remove the cause. In other cases where the bacteria are especially virulent or in exceedingly large numbers, many leukocytes will be killed in the process, with disintegration and dissolution of the dead cells, forming pus."

Toxins and Antitoxins.—Certain bacteria such as those causing diphtheria, tetanus, and botulism elaborate powerful poisons. These are soluble and pass out into the cultural media; hence, they are termed soluble toxins or exotoxins. They are complex organic compounds and possess the following characteristic properties: (1) They are highly labile, that is, they are easily destroyed by adverse conditions, such as drying, free oxygen and oxidizing agents, action of light, and heat if kept at a temperature of 60° C. for a few minutes. The toxins elaborated by the tetanus and diphtheria bacteria are destroyed by proteolytic ferments and do not pass through the uninjured mucous membrane. (2) They produce symptoms of poisoning only after an incubation period. Most poisons, if given in sufficient quantity, may produce almost immediate symptoms. Not so with bacterial toxins, for the injection of even 100,000 fatal doses of tetanus toxin intravenously into rabbits kills only after five hours. It appears that time is required for the toxins to reach and injure the vital tissues. (3) When given in appropriate doses they stimulate the production of antitoxins which neutralize the toxin. If a horse is given a large dose of tetanus toxin, after a certain incubation period the animal dies with the symptoms of tetanus. However, if the horse is given small but increasing doses of tetanus toxin, at cer-

tain intervals it becomes immune to tetanus after a time, and no matter how large a dose is given later the animal develops no symptoms. The blood of such a horse possesses the power of immunizing other animals when given to them.

True toxins are highly potent and injure or kill in smaller doses than other known poisons. It is stated that one cubic centimeter of a broth culture of tetanus toxin is capable of killing from 50,000 to 75,000 guinea pigs, yet the major portion of this broth is water, salts, and peptones. The actual quantity of toxin required to kill one guinea pig is, thus, extremely small.

When heated, toxins lose their power of injuring but retain the property of calling out antitoxins when administered to suitable animals. Such modified toxins are called toxoids. To explain this condition Ehrlich assumed that the toxin has two essential parts: (1) One which combines with the cell receptor, he designated as the haptophore portion. This is unharmed by heat, or light. (2) The other which he named the toxophore portion, is easily destroyed by heat and light, and is the part that poisons the cell. Hence, a toxoid is a toxin in which the toxophore group has been destroyed, and the haptophore group, though intact, still possesses the power of calling out immunity.

If fifteen or twenty fatal doses of tetanus toxin are mixed with an appropriate quantity of horse serum immunized against tetanus and the mixture injected into a guinea pig, it is found to be without effect. The substance present in the blood of the immunized animal and which has been called out by tetanus toxin is known as anti-toxin. It is a substance of complex composition, sensitive to heat, light, and oxygen, and forms a loose combination with its specific toxin, thereby preventing the latter from injuring the animal.

Reaction against Bacteria.—Most bacteria do not produce true toxins; hence, they do not have the power of calling out anti-toxins. However, they do cause the production of other antibodies which destroy either the bacteria or their products. These are known as lysins, agglutinins, precipitins, and opsonins.

Lysins.—Nuttall showed in 1886 that normal blood contained substances which act upon cells, causing them to become granular, then swollen, and finally completely dissolved. These substances are called lysins. Those which dissolve bacteria are known as bacteriolysins; those that dissolve red blood cells hemolysins; and those which dissolve epithelial, or other body cells, are known as cytolysins. These lysins dissolve foreign cells

which enter the blood. This is not always a benefit, for they may in this manner liberate poisonous products. Moreover, their activity in the test tube is often greater than in the body. Nuttall, in one of his early experiments, showed that one cubic centimeter of rabbit's blood contained sufficient bacteriolysin to destroy 50,000 anthrax bacteria, causing their complete destruction in four minutes; yet, according to some authorities 16,000 virulent bacilli will kill a rabbit when intravenously injected. The bacteriolysins are complex substances and readily destroyed by heat. They are specific.

Specific hemolysins may be produced by injecting red corpuscles from the blood of one animal into the circulation of a rabbit. The rabbit then produces hemolysins which react only with corpuscles from the same species as were those injected into the rabbit. That is, if human corpuscles were used to immunize the rabbit, the blood decomposes human corpuscles and no others.

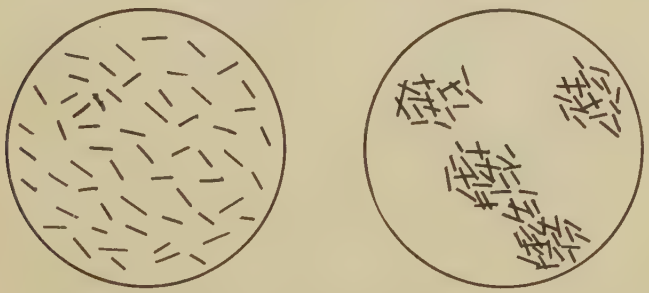


FIG. 93.—Illustrating microscopic agglutination reaction.

This phenomenon has found considerable practical application. (1) It may be used to identify blood. Blood stains are found on the clothing of a suspected man. He claims they are from the lower animals. They are tested against blood having lytic power for human blood and are decomposed. The evidence is conclusive that the stains were human blood. (2) Likewise, it can be used to identify certain bacteria, or to diagnose specific diseases. (3) The method can be used to determine whether the blood of two individuals will mix, and consequently, whether the one can be transfused into the other.

Agglutinins.—When blood of a person immunized against a specific disease (for example, typhoid) is brought into contact with a culture of the typhoid bacteria, it is found that all movement ceases, and that all the bacteria stick together in small

masses or clumps as if some sticky or glutinous substance were holding them together. This is spoken of as agglutination. It appears to be the preliminary change which occurs before the decomposition of the bacteria by the bacteriolysins. It is specific and, therefore, may be used for either the identification of unknown organisms, or the diagnosing of certain diseases and the finding of carriers. The individual who has typhoid fever will give a positive agglutination test when a drop of his blood is brought in contact with typhoid bacilli. This is known as the Widal test.

Precipitins.—Another class of immune bodies which may be produced in the blood are precipitins. They are produced when any foreign protein, bacterial or otherwise, reaches the blood stream. They are similar in most respects to the agglutinins. They have the same values and are used in the practical tests the same as the agglutinins.

Opsonins.—If carefully washed phagocytes are brought in contact with bacteria they take up a few of the bacteria. If to a second batch of the phagocytes some normal blood serum is added and then this is allowed to act on bacteria, it will be noted that the phagocytes take up more bacteria. Now, if immune serum is added to a third batch of the phagocytes it will be found that this third group takes up still more bacteria. Hence, there is something in the blood of normal individuals in small quantities which makes the bacteria more vulnerable to the attack of the white corpuscles. This something is increased in many infections and is known as opsonin.

Theories of Immunity.—Various theories have been evolved to account for the way in which the body overcomes diseases. The two most generally accepted are the cellular and humoral theories. (1) The cellular or phagocytic theory of immunity was formulated by the noted Russian scientist, Metchnikoff, who observed in 1883 that certain cells of the body possess the power of ingesting and digesting bacteria. He and his followers concluded that these so-called phagocytes played the main rôle in the prevention of infection and in freeing the body of it. This theory explains very well the manner in which the body protects itself against many of the parasitic bacteria, but it falls far short of explaining the phenomena in the case of the progressive or true pathogens.

(2) In the humoral theory it is held that immunity is due to substances which result or are produced within the body, primarily in the blood. This theory has been championed by

Ehrlich and his followers and is usually referred to as Ehrlich's side-chain theory of immunity. It is based upon the observed power of tissues to regenerate themselves when injured and to produce an excess of tissue over that necessary to repair the wound. This excess it is held is thrown off and represents the various immune bodies which are observed and known to play a part in immunity.

Fig. 94.—Elie Metchnikoff (1845–1916).

Fig. 95.—Paul Ehrlich (1854–1915).

The cellular theory of immunity was developed primarily by the French, and the humoral by the Germans. At first there was considerable rivalry, each school trying to substantiate its pet theory, but now that the heat of the battle has cleared away, it can be seen that the facts point to the conclusion that in a measure both theories are correct and that immunity must be explained by a compromise or combination of the two theories. It is certain that the phagocytes can ingest and digest bacteria, and it is also true that there are certain substances, opsonins, produced within the blood which render the bacteria more vulnerable to attack. It is also clear that certain substances, antitoxins, are produced within the blood which neutralize bacterial poisons, and still other products, lysins, which decompose them.

CHAPTER XXXII

THE BACTERIOPHAGE

The space as well as the elements which are essential for the growth and normal functioning of plants and animals upon the surface of the earth is limited. Certain plants and most animals are so constituted that they must obtain their food in the form of definite compounds, which necessitates that they receive it in a prepared form from the bodies of other plants and animals. These conditions give rise to a constant struggle for existence. It manifests itself among the members of the same species; even man struggles with man for a place in the sun. Species compete with species, the higher preying upon the lower, on down the line. The little ameba devours the still smaller bacteria. Bacteria standing at the bottom return the compliment, on up the line with a vengeance, they being the greatest destroyers of all. They act as if they were the timekeepers in the race and fight of life, and stand as the link between the living and the dead. When the higher plants and animals have had their borrowed essential elements for their allotted time, the bacteria intervene, end the race, and return the elements to the great earthly reservoir, so that they can be used over and over in the never-ceasing drama of life.

In this scheme of things it appears as if the bacteria stand apart from other forms of life, inasmuch as they have no smaller organisms to prey upon them. Due to their method of multiplication they never grow old; hence, barring accidents, they may be considered as immortal. It is even argued that they were the first living organisms upon this planet, for some of them can subsist upon the inorganic constituents of the bleak rock watered with rain, receiving sufficient energy from the traces of nitric acid which it contains. In the course of evolution, due to differentiation, the higher plants and animals became mortal, thus rendering them liable to natural death and also to disease. While it is true that the single-celled forms of life never grow old and are, therefore, not subject to natural death, bacteria too, may sicken and die, and as some argue, because of living parasites.

History.—Hankin, in 1896, found that the waters of India purify themselves. The Jumna River at Agra contained 100,000 bacteria per cubic centimeter, while three and one half miles below it contained only ninety bacteria. It is a well-known fact that this river is highly polluted, but the disease-causing bacteria quickly disappear from it. This is not due to a poison, for none is to be found in the water. The bactericidal substance is destroyed by heat, as cholera organisms quickly disappeared from the natural waters; yet they actually multiplied in the same water after it had been boiled.

Haffkine, who was in charge of a laboratory in which vaccine against the plague was produced, noted that some of his cultures of the plague bacteria would grow normally for a time, after which they would disintegrate and all the plague-producing organisms disappear. This was such a common occurrence that the workers gave to them the name of "suicides." Why did they commit suicide? If it was a suicide, what was the weapon used in self-destruction?

The first explanation of this phenomenon was offered by Twort, who in 1915 was attempting to cultivate on agar ultra-microscopic organisms from the lumph of a calf. On some of his plates there appeared colonies of micrococci, and in some of these plates "watery looking areas." This apparent diseased condition Twort found could be communicated to other colonies. He made the following interesting suggestions concerning the etiology of this "disease" of bacteria: (1) That the active material might be produced by the micrococcus; (2) that the "virus" might be an ultra-microscopic organism, pathogenic for bacteria; (3) that it might be living protoplasm which formed no definite individuals; or (4) that it might be an enzyme with power of growth.

In 1916, d'Herelle, of the Pasteur Institute, had under observation an adult suffering with a severe case of dysentery. Each day a small amount of the feces was placed in a sterile tube of bouillon. After incubation at 37° C. over night, the growth was filtered through a clay filter which removed all the dysentery bacilli. This clear filtrate was added to a second tube to which had been added previously the dysentery bacilli. This was then incubated, and at the end of twenty-four hours the bouillon was cloudy because of the great number of bacteria which had grown within it. This procedure was repeated daily for some time, when one day, upon examining the tubes prepared the previous

day, there was found to be no growth. When a drop of this was carried to another tube, it was found that growth was prevented in this tube, even though it had previously been heavily seeded with the dysentery bacteria. After considerable work of this nature, d'Herelle suggested that there is in the filtrate a living "ultra-microbe" which develops as a parasite in the bacteria and destroys them. To this he gave the name bacteriophage.

Having learned that there is something which preys upon bacteria, many questions arose, such as: What is the bacteriophage? Where is it found? Is it possible to see it with even the ultra-microscope? Is it a living entity? If it is, what does it feed upon? Can they be counted? Are there different species or races? If so, do they all attack bacteria with the same vigor? Can they be used by man to control the disease-producing microbes?

Source of the Bacteriophage.—D'Herelle first obtained the bacteriophage from the intestines; hence, he gave to it the name *Bacteriophagum intestinale*, and suggested that each new species be designated according to the bacterium on which it acts. Thus

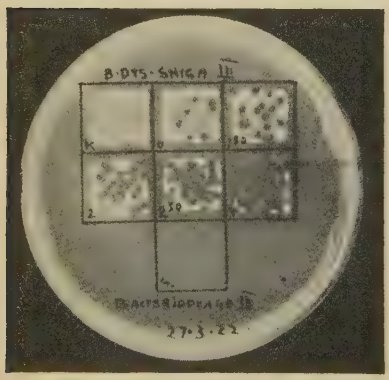


FIG. 96.—Bacteriophage acting on Bact. paratyphosum β. (*After Asheshov.*)

FIG. 97.—Bacteriophage acting on Bact. dysenteriæ flexner. (*After Asheshov.*)

a "shiga-bacteriophage" is one which was originally isolated from a case of dysentery due to *B. dysenteria shiga*, or it is a bacteriophage virulent for this organism. A "staphylo-bacteriophage" was isolated from a lesion caused by the staphylococcus. A

"cholera-bacteriophage" was originally obtained from a case of cholera.

The bacteriophage has been found rather widely distributed in nature. Its chief source, however, seems to be the intestinal tract of man and the higher animals. It is particularly abundant in individuals who are convalescent from a bacterial infection. It has also been obtained from diseased tissues, exudates, bacterial cultures, soil, water, and the like. Being a constant inhabitant of the intestines, it may be encountered in anything which is contaminated by human excreta.

Propagation of Bacteriophage.—The bacteriophage manifests itself only by its action on living bacteria. In reality, the only appropriate cultural media known for its propagation are living cultures of bacteria. So far it has been shown that it can be grown on some twenty different species, among which are both pathogens and saprophytes.

When once a sample of bacteriophage has been obtained, it may be propagated indefinitely by transferring small portions of the culture to a suitable medium containing young cultures of the appropriate bacteria. These must be living and should be young, as it does not multiply on dead bacteria. The medium may be either a liquid or solid, the only requisite being that it be suited to the growth of the specific bacterium which is used in conjunction with the bacteriophage.

If the bacteriophage is seeded into a liquid which is cloudy because of the presence of bacteria, the solution soon becomes clear. The bacteriophage digests completely the bodies of the bacteria, which then pass into solution. If it is seeded on to solid media heavily inoculated with some bacterium that is susceptible to the bacteriophage, it is found that no bacterial growth appears. If the bacteriophage is only lightly seeded, that is, only certain portions of the agar inoculated, these appear as small bare spots, "plagues," on which there is no bacterial growth.

Properties of Bacteriophage.—If a platinum inoculating needle is dipped into a "bacteriophage fluid" and the minute quantity adhering to the wire inoculated into a prepared bacterial suspension, the suspension becomes clear within a few hours; all of the bacteria have disappeared. To all appearances they have dissolved in the bouillon, just as sugar or salt dissolves in water. If a bit of this clear solution is transferred to a second suspension of bacteria, in a short time this also becomes clear. The bacteria

have been digested. This procedure may be repeated a third, fourth, and any number of times, and the final results are always the same. By this method d'Herelle propagated bacteriophage for ten years, during which time it was transferred thousands of times. It is not possible that there could have been anything in the original culture so injurious to bacteria that it could have been diluted to such an extent and still destroy bacteria. Hence, it is concluded that the bacteriophage multiplies.

Those who believe the bacteriophage to be a living entity follow the speed of multiplication and attempt to determine the number. The procedure is very similar to that used in counting bacteria. The number of bacteria in any substance is found by diluting with a measured volume of sterile water. The well-diluted material while in the liquid state is poured into some sterile solid medium. On solidifying, the bacteria become fixed on the surface and grow until they become so numerous that they may be seen with the naked eye— just as a clump of trees can be seen in the valley from the mountain side, whereas a single tree may be overlooked. Probably each group developed from a single organism; hence, if one knows the dilution he can calculate the number in the original sample after counting the colonies.

By seeding measured volumes of the substance to be studied into heavy cultures of bacteria and then pouring onto a solid medium, the bacteriophage can be counted. The bacteria, free from the bacteriophage, multiply and soon become visible, but where the bacteriophage are present the bacteria are prevented from growing; hence, we have a bare spot on the culture medium, and by counting the number of bare spots on the medium, one can ascertain the number of bacteriophage present.

Assuming that bacteriophage are living corpuscles, attempts have been made to measure their size. This is done by passing them through filters of which the size of the pores have been determined. If they pass through one filter of a definite size but not through another just a size smaller, it is known that in size they must be between the two. In this way it has been found that the bacteria are many thousand times larger than the bacteriophage. Bacteria are from 0.1 to 5 microns in length, the bacteriophage from 20 to 30 millimicrons. It requires five hundred million bacteria to weigh one milligram. It would require many thousand times this number of bacteriophage to weigh so much. It has been suggested that bacteria are as much larger than the bacteriophage as a fly is larger than bacteria.

These sizes approximate that of the protein molecule. It seems to leave us in a dilemma. Protein has been looked upon as the basis of life. Is it, thus, possible to have living organisms smaller than the protein molecule? An organism is considered as a cell or an aggregate of cells, and a cell as a mass of protoplasm containing a nucleus. When we learned of the bacterium we came to define a cell as a mass of protoplasm containing nuclear material. It may be that our conception of some cells will have to be modified to read: A cell may be a micella which is composed of material less complex than the protein. Moreover, it has recently been suggested that life may have originated with very simple compounds and not with the complex protein.

The bacteriophage possesses relatively high resistance to temperature; in some cases it is not inactivated when held at 70° C. for twenty minutes. Marked variations have been noted by different individuals. This has caused some to conclude that there is no definite thermal death point for the bacteriophage. D'Herelle explains it as due to different races. He further maintains that all races are completely inactivated in thirty minutes at 75° C. They are very resistant to low temperatures. They are destroyed by ultra-violet light and are sensitive to most chemicals including disinfectants but appear to be more resistant than are bacteria; hence, it is possible to destroy bacteria without inactivating the bacteriophage.

The bacteriophage may adapt itself to its surroundings. Just as Pasteur found it possible to increase the destructive powers of bacteria by successive passage through the bodies of susceptible animals, likewise d'Herelle finds it possible to increase the digesting power of the bacteriophage for bacteria by successive cultivation in the bodies of bacteria. By this means many races have been produced which quickly devour the typhoid, diphtheria, dysentery, and other disease producers. Moreover, these highly active strains are found in the bodies of individuals recovering from these diseases; whereas, when the disease terminates fatally, they are usually absent. Consequently, according to d'Herelle, they are often the determining factors in recovery.

Much discussion has centered around the nature of the bacteriophage. The two leading views are: (1) that it is a living organism, an ultra-microscopic filter-passing microbe; and (2) that it is an enzyme or enzyme-like body produced by bacteria. The first theory has been ably and ingeniously supported by d'Herelle and his followers. Bordet has been just as insistent

in his belief that the lytic principle is of an enzymic nature and is produced by an interaction between the organism and the tissue cells of the host. Final judgment must await further discovery.

Bacteriophage in Immunity.—The discovery of the bacteriophage has caused d'Herelle to offer a modified theory of immunity into which three factors enter: (1) the bacteriophage; (2) cellular immunity; and (3) antitoxin immunity. The bacteriophage acts in three ways: (a) through direct antagonism for the disease-producing agent; (b) by the preparation of bacteria for phagocytosis; and (c) by rendering the bacteria particularly potent as antigens. Thus, the bacteriophage plays a particularly important rôle, for, besides acting directly and independently against the pathogenic agent, it is indirectly concerned in the other two factors. A concrete case will help to give a clear conception of the theory. When typhoid bacilli succeed in entering our tissue, one of four things follows. (1) If we are fortunate enough to be carrying a bacteriophage virulent for the typhoid bacilli, the invading organisms will be attacked and destroyed by it before they have had time to multiply and cause an infection. In this case no disturbance arises, and we remain unaware of the coming and going of the bacilli; theoretically, a degree of immunity would arise as a result of the action of antigenic substances derived from the destroyed bacilli. (2) We may be carrying a bacteriophage that is not actively virulent for the invading bacteria which, unopposed, give rise to infection and disease. In the meantime, the bacteriophage acquires virulence for the infecting bacteria and destroys them before the host is overwhelmed. The disease is thus checked and the patient recovers. (3) The bacteriophage may be able to oppose the bacilli sufficiently to prevent disease but not to destroy them. It is thus that typhoid carriers arise. (4) If the bacteriophage is absent, or present and unable to interfere with the growth of the bacilli, a fatal infection develops.

The Bacteriophage as a Preventive and Curative Agent.—The bacteriophage has been extensively used by d'Herelle in the cure and prevention of numerous bacterial diseases in man and the lower animals. Many of his cures have been little short of marvelous. This has caused some to ask: Will the bacteriophage eventually be developed so that it can be liberated in water and thus free waters of harmful bacteria? Will the physician of the future combat the communicable diseases of man by the use of

virulent bacteriophage which will prey upon the disease-causing bacteria? Will it be possible to make immunity "catching" as is now the case with disease? The claims made by d'Herelle are that we shall. This sounds "too good to be true." Moreover, most workers have failed to confirm his findings. Future work must solve this riddle.

CHAPTER XXXIII

ANTITOXINS AND VACCINES

Microbial infection is nothing more or less than an immunizing contest between a host and an invader. Each contestant is feverishly engaged in manufacturing products with which to destroy its opponent or to render itself immune against the weapons of its enemy. Hence, the outcome of the struggle is determined by the quality and quantity of the defensive and offensive products produced by the contestants. Any advantages which one can gain over the other often determines the outcome. Man is learning how to supply the host with defensive weapons in the form of antitoxins and how to stimulate the cells of the host by vaccines to a rapid production of both offensive and defensive weapons before he is attacked by his microbial enemy.

Historical.—It was Frederick Löffler, a student of Koch, who discovered the bacillus which causes diphtheria, and Emile Roux, a pupil of Pasteur, who demonstrated that in this disease the organism grows at the site of inoculation and elaborates a toxin which is distributed by the blood and injures the tissues. It was found that by growing some of the bacteria in bouillon, filtering off the germs, and injecting the clear filtrate into the tissues of susceptible animals, diphtheria resulted. Through further experiment on guinea pigs another student of Koch, Emil Behring, succeeded in showing that recovery from the disease is due to the production of antitoxins which counteract the poison in the blood. By 1895 Behring and others had so far perfected a method of producing antitoxin on a large scale that it could be used in the treatment of diphtheria. From that day to this the diphtheria death rate has steadily declined. The diphtheria rate per 100,000 population has dropped in New York City from 110 in 1895 to ten in 1925. During the same period the mortality has decreased from thirty-five to seven, and of those who receive antitoxin treatment on the first day of their illness the mortality is less than one per cent.

Nature of Antitoxins.—Antitoxins are antibodies capable of directly and specifically neutralizing toxins that cause their pro-

duction. There are both animals and plants which produce toxic substances possessing the power of calling out antitoxins. The venom of the rattlesnake and cobra, the poison of the scorpion and the tarantula, the poisons produced by certain fishes and insects are examples of animal toxins. They are known as zoötoxins. Toxins produced by plants (phytotoxins) are found in the juice of the castor oil bean, ricin, the bark of the locust tree, robin, poisonous mushrooms, and many other plants. Only a few bacteria produce true toxins. The principal ones are the diphtheria, the tetanus, and the botulinus bacilli, and probably certain forms of the dysentery bacilli and *B. pyocyaneus*.

Although antitoxins are more resistant to heat and chemicals than are the toxins which cause their production, yet boiling, putrefaction, and many chemicals destroy them. They are complex organic compounds associated with the serum globulin and are possibly proteinaceous in composition. All attempts to separate them from the blood proteins have so far failed. Hence, a more accurate designation of the biological product is antitoxic sera and not just antitoxin. In the serum of the blood of animals immunized to the true toxins the main defensive product is antitoxin. There are usually present other antibodies, lysins, opsonins, precipitins, and agglutinins, which assist in overcoming the invading organism. Antitoxins are recognized by their power of neutralizing toxins with which they apparently combined and render non-toxic. They are produced in the bodies of animals, as a result of the entrance of a toxin; consequently, each antitoxin has a corresponding toxin that causes its production.

Inasmuch as many individuals fail to produce the appropriate antitoxins or else produce them in inadequate quantities (and hence would die or suffer irreparable damage from the attack), modern therapeutics forestall these failures on the patient's part by supplying him with the antidote from another source. That is, the patient is given an antitoxic sera which contains the appropriate antitoxin for neutralizing the toxin produced by the invading organism. In other cases the cells of the individual are trained before hand to produce the needed defensive substances by the use of an appropriate vaccine. The latter is nothing more or less than a mild attack of the disease.

Preparation of Diphtheria Antitoxin.—Diphtheria and tetanus antitoxins are manufactured today on a large scale and are extensively used in the prevention and cure of these diseases. But, inasmuch, as antitoxins are produced in the body of an

animal only by the stimulation of a toxin and then transferred to a second animal, it is necessary first to consider the preparation of the toxin.

Toxin Production.—Diphtheria toxin is produced by seeding the germ in large flat bottom flasks containing a relatively thin layer of a specially prepared nutritive broth. For the best results the media must be prepared with great care from fresh lean beef which has been freed from muscle sugar by fermentation with *Bact. coli.* It is essential that the diphtheria organisms receive an abundance of oxygen and be grown at a temperature of from 35° to 36° C. for maximum toxin production. The growth spreads over the surface of the broth, and in from seven to ten days the container is removed from the incubator, tested microscopically for purity, and a little carbolic acid added to kill the bacteria. After forty-eight hours the dead bacilli have settled to the bottom. The clear liquid is then siphoned off and filtered through a porcelain filter to remove any bacterial bodies which may be present. The strength of the toxin is determined by injecting carefully measured quantities into guinea pigs weighing 250 grams. The smallest quantity that is sufficient to cause the death of such a test animal in a given time is taken as the unit and is known as the minimum lethal dose, "M.L.D." Knowing this, the manufacturer of antitoxin can ascertain with certainty how much may be used with safety on the animal which is to be used for the preparation of antitoxins.

Preparation of Diphtheria Antitoxin.—In the preparation of diphtheria antitoxins the horse is used. This animal is used in this connection for several reasons: (1) It produces large quantities of antitoxin. (2) Because of its size, considerable quantities of blood may be taken without permanent injury. (3) It produces a blood, the proteins of which are borne better by man than is the blood from other appropriate animals. Young, vigorous, absolutely healthy horses of fair size are used. Every precaution is observed to see that they are free from disease. Generally, it is the practice to give first some antitoxin, and on the following day to inject a small quantity of toxin under the skin. Usually for a day or so the horse refuses to eat and there is some fever. As soon as the fever subsides, usually in two or three days, a second and larger dose of toxin is given. This is continued for seven or eight doses, each dose being slightly larger than the preceding one. Smaller quantities are then given until the maximum antitoxic content of the blood is

reached, which occurs only after several months and can be determined by bleeding the horse and testing the blood against a standard toxin. There is no known way of telling beforehand whether a horse is going to produce a strong antitoxin or only a weak one. When a horse is found which has the power of producing a highly potent serum, it is used for long periods for the production of antitoxin.

When the blood of the horse reaches a high antitoxic content, the horse is bled by introducing a hollow needle, or cannula, into the right jugular vein, and the blood is allowed to flow into a

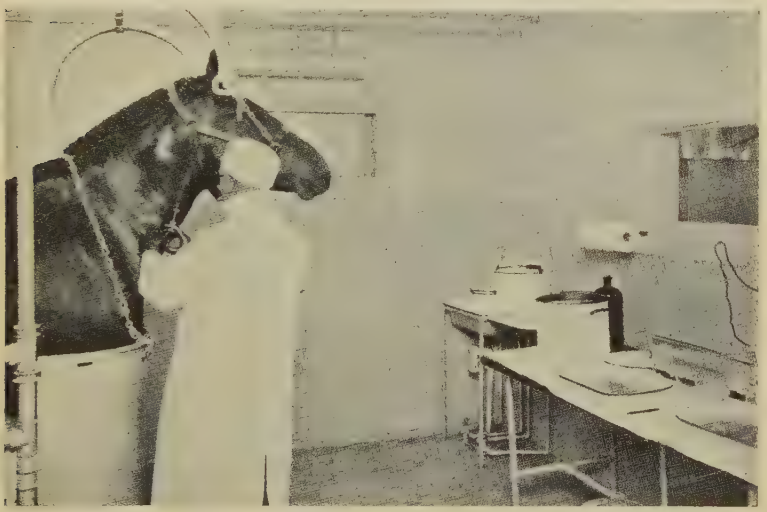


FIG. 98.—Injecting serum horses. (*Courtesy of Parke, Davis and Co.*)

sterile jar. The cannula, tubing, jar, and everything used in collecting the blood and serum are carefully sterilized, and the whole operation is conducted with the greatest of aseptic care so as to avoid contamination. From 6 to 12 liters of blood are removed. The horse is then permitted to regain its strength after which the injection of toxin is again commenced, and after a time the bleeding is repeated. In a recently proposed method, the blood is collected into an oxalate solution, the corpuscles separated from the plasma and reinjected into the horse. This prevents anemia and other bad effects which follow the too-frequent removing of large quantities of blood.

The jars containing the blood are placed in a refrigerator for a few days, during which time the blood clots, and the clear serum collects on the surface. The latter is siphoned off and passed through porcelain filters to free from any solid particles including bacteria which may have gained access to it. The serum should be clear and free from blood. Its sterility is determined by microscopic and cultural tests while its strength is determined by mixing accurately measured quantities with a toxin of known strength and injecting this into guinea pigs of standard weight. Often the antitoxin is concentrated by definite chemical procedure

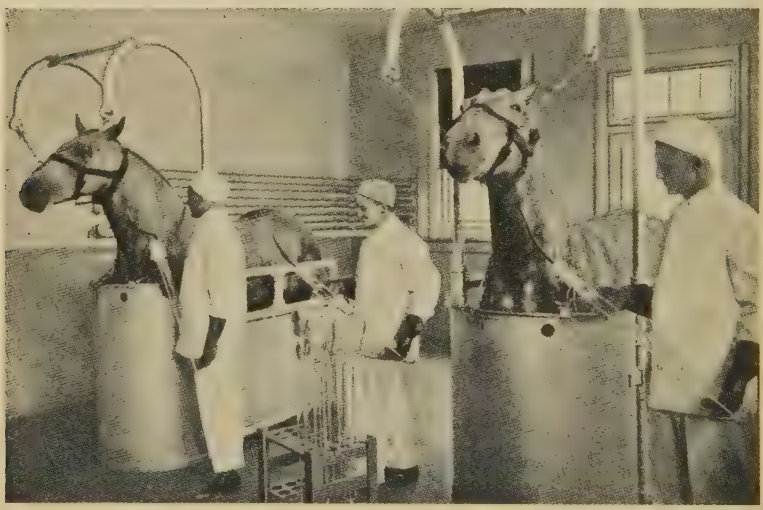


FIG. 99.—Bleeding antitoxin horses. (*Courtesy of Parke, Davis and Co.*)

before standardizing, so as to remove much of the objectionable protein which tends to cause serum sickness in the recipient. When the serum is to be kept for some time a small quantity of an antiseptic is added, after which it is dispensed in receptacles, usually closed syringes, ready for use. It deteriorates more rapidly at high than at low temperatures; hence, it should be stored at a low temperature. Most laboratories place the date after which it should not be used on the label. Not that it becomes injurious on standing, but it loses strength to such an extent that after a time it cannot be depended upon as a curative or preventive agent.

Antitoxin is used extensively as a curative agent, and if given to the patient on the first day of the disease usually effects a cure. Later use is not as successful, for the diphtheria toxin rapidly enters the tissue and does its damage. Where paralysis follows a case of diphtheria in which the antitoxin has been given, the injury results from the disease and not from the antitoxin. The antitoxin may have been given too late or in too small a quantity; consequently, the toxin was not neutralized. The antitoxin itself is harmless. In some cases serum sickness follows the injection of the foreign protein into the blood stream. This is usually only mild and caused by the blood proteins and not by the antitoxin. Antitoxin is also used as a preventive where individuals have recently been exposed to diphtheria. However, where a lasting immunity is desired, a mixture of toxin and antitoxin is given. This mixture is such that the injurious action of the toxin is neutralized, but still possesses the power of stimulating the body to produce its own antitoxin. Three doses are given, one week apart, and the child develops an immunity to the disease which lasts for many years and probably for life. This method was first used in 1913, and the results have been so encouraging that some authorities claim that diphtheria could be prevented entirely if every child were given the treatment by the time it is nine months of age.

Other Antitoxins.—When the great curative and preventive value of diphtheria antitoxin was discovered great hopes were entertained that the same principle could be applied to other diseases, and we only needed to await the time when the millenial hope of a diseaseless world would occur. However, this hope has been satisfied in few other cases. Diphtheria antitoxin is still the outstanding example of a real curative serum. Tetanus antitoxin is the only other antitoxic serum which even approaches this hope. It has high protective but low curative value. Antitoxins have been prepared capable of neutralizing the toxin of the botulinus organism and snake venoms. These are manufactured in essentially the same manner as the diphtheria antitoxin.

Vaccines.—Vaccines are used against a number of diseases. They cause a mild attack of the disease which is brought about by the use of an attenuated or killed culture of the virus which neither threatens the individual's life or health, nor the life and health of his associates. The principal methods by which this is accomplished are as follows: (1) By the use of living attenuated cultures. The cultures used are attenuated by

various means: (a) by growing in the body of other animals; (b) by growing on certain laboratory media or at abnormal temperatures; (c) by heating, or growing at abnormal pressure, or in the presence of certain chemicals. To this group belong the vaccines for smallpox, rabies, anthrax, and many others. They have lost their power of producing a typical attack of the disease but still possess the power of calling out immunity. (2) By the use of dead bacteria and bacterial extracts. The germs are grown in laboratory media and then killed by heat or chemicals, and their bodies or extracts of their bodies used to immunize the animal. This is the method used in typhoid immunization. (3) Active immunization may be produced by the use of small doses of fully virulent cultures of the pathogens. This method, due to its possible dangers, is used primarily in veterinary medicine. (4) Active immunization can be produced in some diseases by the use of the bacterial products, toxins. The best example of this is the immunization against diphtheria by use of the toxin antitoxin.

Smallpox Vaccine.—The first, and most successful use of a vaccine for the prevention of a communicable disease, was in the case of smallpox. The term "vaccine" originated from the term *vacca*, meaning a cow, but it is now used in a broader sense. In the early days of smallpox vaccination it was the custom to vaccinate one individual from the pustule on the arm of another. The old-time physicians saved the scabs from typical vaccination cases and carried them about in suitable containers ready to vaccinate their patients. There are two great objections to this method: (1) The supply was limited and was collected by various individuals without proper sanitary care. (2) It was possible in this way to spread human diseases, especially syphilis.

For many years it has been the custom to propagate the vaccine in young healthy animals. This obviates all danger of human disease and makes it possible to prepare a uniform virus under carefully controlled sanitary conditions. Various animals have been used. Female calves from two to four months of age are preferred, because they are easily managed and have such a tender skin that the eruption formed on it is abundant and typical. Furthermore, they can be kept on a milk diet, thus obviating the dust which is associated with the use of fodders. The animals are held under observation in quarantine for seven days to determine the presence of any infectious

disease and are then thoroughly cleaned before vaccinating. The belly and flanks are clipped, and the skin is thoroughly washed with soap and sterile water. Each animal is then vaccinated by making about 100 small scarifications and carefully inoculating these with the seed vaccine which has been prepared in the following manner: "Crusts are collected from healthy children about nineteen days after successful vaccination. These crusts are cut up and emulsified with boiled water to a mucilaginous paste. This humanized seed is inoculated into an area about

FIG. 100.—Removing smallpox vaccine. (*Courtesy of Parke, Davis and Co.*)

six inches square upon the abdomen of a calf, the remainder of the calf being vaccinated in the ordinary way. The pulp from this special area is separately collected and glycernized in the usual way. It is then tested bacteriologically and clinically. This bovine virus from human seed is now used in a dilution of 1 part to 12½ parts of normal salt solution to vaccinate rabbits. The seed is rubbed thoroughly on the freshly shaved skin of the back. Five days after vaccination the pulp is removed with a curet, weighed, and emulsified in a mortar with the following solution: glycerine 50 per cent, sterile water 49.5 per cent, and carbolic acid 0.5 per cent, in the proportion of 1 part of pulp to 8 parts of the solution. Four rabbits should yield from 15

to 20 cc. of this emulsion, an amount sufficient to vaccinate one calf."

The animals are kept in clean stables, and in 5 or 6 days the pustules which have formed at the site of the vaccination are ready for the collection of the virus. The calf is killed, the vaccinated area thoroughly cleaned, and the vaccine collected under as rigid aseptic conditions as are used in a major operation. The vaccine is mixed with glycerine and allowed to stand for three or four weeks so as to destroy bacteria which may have gained entrance. Its strength is measured by inoculating into rabbits. Its bacterial content is determined by plating and inoculating into guinea pigs. The calf from which the vaccine was collected is carefully examined by a veterinarian, and if any signs of disease are found the entire batch is destroyed. After it has met all careful laboratory tests, and not until then the vaccine is submitted to a final clinical test on unvaccinated individuals, after which it is placed in fine tubes, or on points and is ready for distribution. The yield of vaccine varies greatly with calves; some yield only sufficient for 500 vaccinations, whereas others yield sufficient for ten thousand. It should be kept at a low temperature (35° to 40° F.). Often failures in vaccinating individuals successfully are due to the deterioration of the virus which may have been kept in a warm place. From what has been stated it may be seen that every precaution known to man is used to insure a safe reliable vaccine, with the result that it can be used knowing that one is endangering neither life, nor limb, and that it will absolutely prevent smallpox epidemics.

Rabies.—This is a disease transmitted by the bite of a rabid animal. Until the time of Pasteur it had a mortality of practically 100 per cent. Today when properly treated it has a mortality of less than one per cent. However, to be successful, the Pasteur treatment must be given before the onset of the symptoms. The virus is usually attenuated by drying and less often with chemicals.

Pasteur found it necessary to first obtain a fixed virus. This he did by taking some of the nervous tissue of a rabid dog and injecting it into a rabbit. Nerve tissue is obtained from this rabbit and inoculated into a second and so on until it has passed through some 30 to 50 rabbits. By this time the virus has been so intensified that it kills a rabbit in from six to eight days. This is referred to as a fixed virus and is inoculated into another rabbit. This animal is kept under the best of sanitary condi-

tions and just before its death is chloroformed, and the nervous cord removed under aseptic conditions. The cord is cut into pieces, and dried over an appropriate drying agent—parts of it for one, other parts for three, and so on up to eighteen days, after which it is mixed with water and given hypodermically to the patient. The pieces of the cord which have been dried longest, from 8 to 18 days, depending upon the method used, are given first, followed at daily intervals by the cord which has been dried for a shorter time, so that eventually the individual is receiving cord which has been dried for only one day.

Typhoid Vaccine.—In vaccination against typhoid the bodies of the dead bacteria are used. The organisms are grown on agar, and after twenty-four hours are washed with sterile normal salt solution, standardized by counting the number of organisms in a given quantity, and then killed by heating to 56° C. for one hour. As a matter of safety 0.25 per cent of tricresol is mixed with it. In order to immunize individuals against typhoid fever, three injections are given at intervals of one week. The dose used in the army has been as follows:

First dose	500,000,000 bacilli
Second dose	1,000,000,000 bacilli
Third dose	1,000,000,000 bacilli

Although it is not an absolute protection against typhoid, yet its practical value was amply demonstrated in the World War.

Tuberculosis.—Most individuals who live in crowded communities become infected with tuberculosis during the first 15 or 20 years of their life; consequently, the adult possesses considerable immunity to the disease. Rarely is it possible to form an opinion as to when the infection took place, and in no single instance has it ever been possible even to guess at the size of the infecting dose. Truly, this is a most haphazard vaccination, yet so effective is it that only one out of every ten infected succumbs to the disease. This has caused various students of the subject to ask: Is it not illogical to permit this chance infection when it is those who receive the excessive doses who succumb? Is it not possible to carefully control the dose and obtain the immunity without the dangers which accompany the natural haphazard method? Some investigators have attempted to answer those questions by experiments on cattle. They have used very small and increasing doses of the living bacilli with

what appears to be promising results. Also a few carefully guarded and conducted tests have been made on human beings who were living under conditions such that natural infection was inevitable. The results have been very encouraging. However, there has as yet been no one to seriously advocate universal vaccination with living bacilli. The nearest approach to it are the experiments being conducted by Calmette and co-workers in France. They use a bacillus attenuated by thirteen years' growth on a bile medium. The organism, so they claim, has completely lost its power of causing disease but retains its power of producing immunity. They claim, as can be seen by the following quotation taken from Long, that this is the only rational method to produce immunity in tuberculosis. "Immunity to tuberculosis can be produced only through the use of living tubercle bacilli. These bacilli must retain the essential characteristics of true tubercle bacilli, viz., acid-fastness, toxicity for tuberculosis subjects, and the ability to produce tuberculin. To be of any value at all vaccination must be practiced before infection from elsewhere has occurred, which for practical purposes in the usual environment of the experiment, is at present conducted, means within the first ten days of the infant's life. The vaccination of infants is best carried out by oral administration; experiments have proven conclusively that in the first few days of extrauterine life the intestinal mucosa is readily permeable to bacteria. The live bacillus used, however, in Calmette's opinion should be so far attenuated as to have lost permanently the capacity to produce anatomical tubercles in susceptible animals." Even then its use is advocated only on infants who must live under conditions such that early infection is inevitable. Some 10,000 children have already been vaccinated in France, but it is too early to draw definite conclusions as to its exact value. However, if it meets the expectation and claims of its originators, it will be one of the great advances which have been made in preventive medicine, and the medical dream of a diseaseless future will have moved one notch nearer its realization.

CHAPTER XXXIV

THE PYOGENIC COCCI

There is a group of parasitic bacteria constantly inhabiting the exposed surfaces, skin, and mucous membranes of man and the lower animals. They are "opportunists" lying in wait for any break which may occur in the outer defenses. Ordinarily they do not force their way through the healthy unbroken coverings of the body, but when these are damaged by mechanical, chemical, or even biological means they invade and do slight or even fatal damage, depending upon the host and the virulence of the invader. Having entered the tissues of the body they are ordinarily unable to escape in sufficient numbers to cause progressive disease of like nature in the tissues of other animals. They are locked up as it were and perish, but when conditions are made optimum for their spread from host to host, as it often was before the days of aseptic surgery, their virulence increases. Hence, they spread through the hospitals and became the great destroyer of their inmates and the terror of the surgeons. They cause inflammation followed by a purulent exudate commonly referred to as pus; hence, their name "pyogenic." Although many organisms possess this property, yet the most important members are either staphylococci or streptococci. There are different varieties or races of each of these, the type species being *Staphylococcus aureus* and *Streptococcus pyogenes*, each of which we shall now consider more in detail.

Staphylococci.—The staphylococci are quite widely distributed in nature. The parasitic forms are usually found in association with man or the lower animals. They are occasionally found in the dust of houses, and occur in hospitals if proper precautions are not exercised. They are common upon the skin, the nose, mouth, eyes, and ears of man. They are nearly always present beneath the finger nails and sometimes occur in the feces, especially of children. They are common in many skin eruptions such as "blackheads," boils, superficial, and deep abscesses. They are the most common organism found in suppuration in man and the lower animals, as the following table from Karlinski shows:

		Cases
Suppuration in man	Staphylococci	144
	Streptococci	45
	Other bacteria	15
Suppuration in the lower animals	Staphylococci	45
	Streptococci	23
	Other bacteria	15
Suppuration in birds	Staphylococci	40
	Streptococci	11
	Other bacteria	20

In general, it may be stated that the lesions which they produce tend to be circumscribed rather than diffused. This is, of course, subject to exception, for they may at times cause a generalized condition. They do not appear to be adapted to a purely saprophytic existence and are usually associated rather closely with man and the lower animals, living a parasitic life.

Morphology.—The name "staphylococcus" has been given to this group of bacteria because of their microscopic appearance; since they commonly occur in masses which have a somewhat fanciful resemblance to bunches of grapes. *Staphylococcus pyogenes aureus* is the most important member of the group. The organism when occurring isolated is spherical, but when in groups has its adjacent sides somewhat flattened. It generally occurs in irregular masses but may occur singularly or in pairs and rarely in chains of four to six cocci. Its average size is from 0.7 to 0.9 micron, but occasionally either larger or smaller forms occur. Usually the larger cocci are those about to divide. It stains well with the basic aniline dyes. In fresh specimens from an acute sore many of the leukocytes will be found to contain within them one or more cocci. They are non-motile and do not produce endospores.

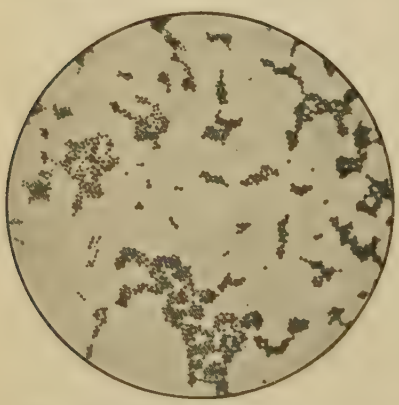


FIG. 101.—Staphylococcus aureus. Fuchsin; ×1000. (*Günther.*)

Physiological Properties.—Staphylococci grow well on all common laboratory media and at a temperature between 12° and 43° C., the optimum being about 37° C. The aureus pro-

duces a golden-yellow pigment which is especially profuse upon blood serum, or upon a starchy medium such as potatoes, or rice. However, even a good pigment-producing strain, when kept on laboratory media for some time, gradually loses its color properties. *Staphylococcus albus* produces no pigments, the growth being of a whitish color, whereas *Staphylococcus pyogenes citrus* produces a bright lemon-yellow pigment in culture. The two latter are much less pathogenic than is the aureus. Some strains are almost completely non-virulent.

The staphylococci are more resistant to adverse conditions than are the majority of non-spore-producing pathogens. They are killed only after comparatively long exposure to bright sunlight. Indirect daylight may fail to destroy their vitality even if permitted to act on these organisms for as long as two weeks. They retain their vitality during months of drying and are very resistant to low temperatures. When dry they may require a temperature of 90° C. to kill, but when moist, a ten minute exposure of 60° C. is usually fatal. Chemical disinfectants require either longer time or stronger solutions to render these organisms harmless, than are necessary for the majority of non-spore-forming bacteria.

Pathogenicity.—Although *Staphylococcus aureus* is pathogenic for both man and the lower animals, all the evidence points to the conclusion that man is more susceptible to it than the lower animals. These organisms ordinarily enter the tissues only after the outer protective coating has been injured in some manner, but a number of experimenters have been able to infect themselves by rubbing pure cultures on unbroken skin. They give rise to acute boils and carbuncles and, generally speaking, to acute focal inflammations which may be mild or severe, depending upon the virulence of the organisms and the tissues in which they lodge. When they locate in or on the bones they cause very serious diseases known as periostitis or osteomyelitis, depending upon the part of the bone attacked. When they reach the lungs or heart, they may, under appropriate conditions cause diseased conditions of these organs. Staphylococcus infection of the lungs sometimes occurs, and, the resulting pneumonia is highly fatal. In the epidemic of influenza which swept the country in 1918–1919, this was frequently the cause of the fatal termination. This organism is often a secondary invader in pulmonary tuberculosis, diphtheria, and other severe infections. Occasionally, it may enter the blood and give rise to septicemia.

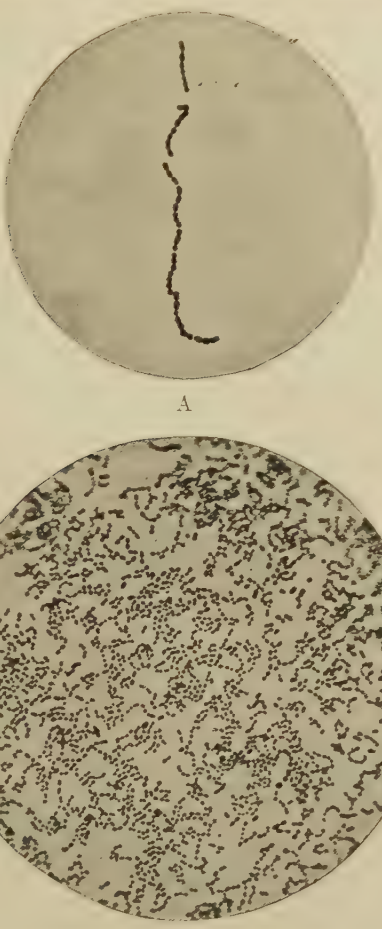
Immunity.—The immunity of man to the staphylococci is usually high and generally they enter only after the natural defenses have been broken down. Whenever the continuity of the skin and mucous membranes is destroyed, as by abrasions or weakened as in diabetes and nephritis, they invade the underlying tissue. This causes an inflammation with the congregating of the white corpuscles at the point of the attack. In most invasions the phagocytes quickly free the tissue of the invader. The immunity is probably due in a great measure to the phagocytes; but it is short-lived and may even be localized, as a patient recovering from staphylococci infection on one part of the body may at the same time have a new infection occurring in another part.

Streptococci.—Streptococci are rather widely distributed in nature. They occur under the same conditions as do the staphylococci in intimate association with man. They are to be found on every square inch of the skin and possible even more abundantly on all exposed mucous surfaces. They are common in the mouth, nose, throat, and the intestinal tract. They also occur in soil, water, and milk. The distribution and virulence varies with the different groups. Holman lists the habitat of the five different groups which he recognizes from reactions in certain carbohydrates and upon blood as follows:

1. *Streptococcus pyogenes*—nose, throat, blood
2. *Streptococcus anginosus*—nose, scarlet fever, endocarditis, epidemic sore throat, septicemia
3. *Streptococcus viridans*—nose, throat, endocarditis, rheumatism
4. *Streptococcus fecalis*—feces, milk, rheumatism, endocarditis
5. *Streptococcus salivarius*—throat, milk, pyorrhea.

Morphology.—Streptococci consist of spherical or oval cells attached and forming chains. The number of cells in a chain is subject to great variations, usually running from four to twenty. Strains having eight or more in a chain are referred to as *Streptococcus longus,* shorter chains as *Streptococcus brevis.* The longer chains are usually obtained from suppurating lesions, whereas the shorter occur more generally upon the healthy surfaces of the body. Attempts have been made to show a relationship between the pathogenicity of the organism and the length of the chain. This has not been very successful as, at least within certain limits, it varies with the cultural media. The individual cells vary from 0.5 to 1 micron in diameter. Larger cells which are quite com-

mon in old cultures and were formerly considered to be a form of spores are called arthrospores. It is now known that they are no more resistant than the normal cells and are generally con-



A

B

Fig. 102.—Streptococci. A, (*From Kolle and Wassermann*) B, (*From Moser and V. Pirquet*).

sidered involution forms. The streptococci are non-motile without flagella and do not form endospores and stain readily with the ordinary analin dyes.

Physiological Properties.—The streptococci grow readily on the ordinary laboratory media, but not so well or luxuriantly as the staphylococci. They are either aërobes or facultative anaërobes. Growth occurs between 15° and 45° C., the optimum for *Streptococcus pyogenes* being 37° C. They are killed in one hour when kept at a temperature of 60° C., except when in a proteinaceous medium. They may retain their virulence for a few minutes at a temperature of 100° C. in dried sputum, and at ordinary temperature they will survive for days, or even weeks. They are quickly killed when exposed to direct sunlight but survive for some time in blood and similar media. The ordinary disinfectants are effective against them.

Considerable work has been done on the classification of the streptococci. They have been divided into groups according to their action upon carbohydrates and blood pigments. However, Rosenow and co-workers claim to have been able to transmute seventeen strains of *Streptococcus viridans* into pneumococci and twenty-one strains of hemolytic streptococci into viridans and then into pneumococci, such changes having been brought about by variations in cultural conditions and by animal passage. The cultural changes included reaction, temperature, oxygen tension, salt concentration, and growth in symbiosis. This would seem to bring into question the stability of bacterial species, but Rosenow's interpretation of the phenomenon is not generally accepted by bacteriologists.

Pathogenicity.—Man is very susceptible to invasion by the *Streptococcus pyogenes*, and once it passes the outer defences there may result local, or even generalized infection. However, their power of invading varies greatly with the different strains. Those from infected lesions, and especially those which have passed through the body of several animals, have greater invasive power for animals of the same species than those which have been growing on healthy tissue. The organism may cause local inflammatory and suppurative processes and even generalized septicemia. Streptococci cause erysipelas and are often the cause of peritonitis, meningitis, and tonsilitis. They often play a prominent rôle in pneumonia and in tuberculosis. They are greatly feared in the cases of osteomyelitis and puerperal fever. They have given rise to numerous epidemics of septic sore throat. At one time it was believed that this disease was of bovine origin, but it is now generally conceded that the streptococcus organisms causing it come from the throats of the milker. This does not,

however, exclude the possibility of the streptococci being accidentally transferred to the udder of the cow and for a time even giving rise to a disease in this animal, and secondarily in man. They often occur in the throat during measles and may cause pneumonia. They are secondary invaders in diphtheria and many gastro-intestinal diseases, and play a prominent rôle in the diseases of the lungs, particularly in the pneumonia which follows influenza. "Thus Rosenow and his co-workers have shown from the standpoint of etiology that streptococci are not only present in rheumatic fever, and in endocarditis, pericarditis, and myocardial lesions, as well as the joints affected in the disease complex, but also in such apparently diverse affections as appendicitis, ulcer of the stomach and duodenum, cholecystitis, eruthema nodosum, herpes zoster, mumps, myositis, iritis, iridocyclitis. Each strain of *Streptococcus viridans*, isolated from one of these conditions, will on injection intravenously into rabbits or dogs tend to localize in the same tissue and produce the same lesion in a relatively large percentage of animals as compared with the percentage of such lesions in animals inoculated with non-specific strains of streptococcus."

Importance.—Before the days of aseptic surgery, infection, caused by the pyogenic bacteria, ran riot in hospitals. Even minor operations were usually followed by suppuration, and in major operations pus formation was so profuse that the life of the patient was always endangered. Most wounds of the abdomen were fatal, and a prudent surgeon shrank from major operations on either the head or the abdomen. We now know that the trouble came from the carrying into the tissue pyogenic bacteria, for once they gain entrance, fever, abscesses, blood poisoning, gangrene, erysipelas, one or all, may start up into ominous and fatal activity. Puerperal or "childbed fever," which is due to streptococcus, used to kill one-half, two-thirds, or even three-fourths of the women in the maternity wards. According to Williams: "In 1866 Lefort showed that in 888,312 obstetric cases in the hospitals of France up to 1864, 30,394 women had died of puerperal fever; that is to say, 3.5 per cent, or about every twenty-seventh mother. From 1860 to 1864 the maternity mortality of Paris had risen nearly four-fold, to 12.4 per cent. In December, 1864 it rose to 57 per cent; that is to say, more than one-half of the women who bore children in that hospital in that month died of childbed fever." Similar conditions existed in other civilized countries. Hence, it is evident that an enor-

mous toll of human life was being taken by these microbes. With the development of aseptic surgery these losses have been checked, but there are still numerous mild and many severe afflictions which are definitely known to be caused by the pyogenic bacteria. Furthermore, if we accept the theory of focal infection and its effect upon the human body, we shall have to conclude that the pyogenic cocci are still the greatest destroyers known to man.

Briefly stated, the theory of focal infection and its relation to human ills is as follows: Focal infection is a circumscribed area of tissue infected with disease-producing microörganisms. This may be located in any part of the body, but the most common foci of infection are the teeth, tonsils, sinuses, gastro-intestinal, and gastro-urinary tract. Having gained a foothold in the tissues the microörganisms multiply, elaborate their poisons, and find their way into the lymph and blood streams. These distribute them to all parts of the body, and wherever they find suitable conditions they locate and cause diseased conditions. Hence, the vicious cycle for sore throat is: tonsillitis, rheumatism, heart disease, and finally death. That is, according to the doctrine of focal infection, an infected tooth, or a diseased tonsil is like an octopus, capable of stretching out its tentacles to such remote organs as the gall bladder, kidneys, heart, and all the vital organs of the body. Therefore, when bacteria invade and multiply in certain tissues the infection becomes a potential menace to all other tissues of the body.

Some of the diseases enumerated by Billings which may result from focal infection are:

1. Various forms of rheumatism: rheumatic fever (with various types of heart involvement) and rheumatism of the joints, muscles, and other tissues.
2. Acute chorea, commonly called "St. Vitus's Dance," characterized by abnormal muscular movements, chiefly by twitchings, as well as nervous and mental disturbances.
3. Malignant or ulcerated endocarditis, a disease of the lining of the heart, having a high mortality.
4. Nephritis, acute or chronic kidney infections with abnormal urines.
5. Acute appendicitis.
6. Cholecystitis, acute or chronic infections of the gall bladder.
7. Gastric and duodenal ulcer, acute or chronic stomach sores.

8. Acute or chronic pancreatitis, an affliction of the pancreatic gland.

9. Erythema nodosum, a skin disturbance frequently associated with rheumatism.

10. Herpes, "shingles," or skin eruptions caused by nerve involvement.

11. Spinal myelitis, an affliction of the spinal cord influencing gait and causing dizziness, or unconsciousness.

12. Acute osteomyelitis, an affliction of the bone marrow usually occurring in the extremities.

13. Thyroiditis, swelling and tenderness of the thyroid gland.

14. Iridocyclitis an affliction of the iris of the eye.

The evidence in favor of this theory is experimental laboratory work and the accumulated clinical experience of many medical men extending over a period of years. For example, Rosenow, in the different types of rheumatic fever has always found at the proper stage of the disease in the joint exudate, joint capsule, circulating blood, tonsils or alveolar abscess, or other foci, certain strains of streptococcus. These, when injected into rabbits, under appropraite conditions, produce the typical disease. Likewise, streptococci obtained from individuals with kidney disease, if given to suitable animals, cause infections of the kidneys. Even bacteria obtained from an infected appendix if given other animals cause a diseased appendix such as that from which the bacteria were isolated. This same method has been applied to the other diseases with similar results. These findings have been confirmed by some, whereas other workers using the same technic have obtained diametrically opposite results.

The bacteriological phase of focal infection is thus summarized by Kopeloff:

"First: certain bacteria have a special affinity for certain bodily tissues. Secondly: the members of the streptococcus-pneumococcus group are closely related and transmutable. Thirdly: streptococci having a special affinity for certain tissues may be isolated from other bodily organs, the teeth and tonsils being especially favorable sites, from which further specific infections may be set up in remote organs. In the last analysis, therefore, the doctrine of focal infection depends upon certain important bacteriological findings. How valid are they?"

Granting that focal infection plays an important part in the afflictions of man, its significance becomes obvious if we consider a few of the diseases in which it is an important factor.

Effects of Focal Infection.—Heart disease is now first on the list of the causes of death. It is also first from the amount of damage it does in producing disability and invalidism, for nearly two hundred thousand persons die from it annually in the United States. If the present situation continues unchecked, one in every five of the population now living will eventually succumb to this disease. Although the greater number of the deaths are among those past the prime of life, yet many young from all walks of life are numbered among its victims.

It not only kills but it incapacitates. It is estimated that for every death from heart disease that occurs annually there are probably ten persons living impaired and deficient lives because of the breaking-down of the heart function. This would mean that there are approximately two million people in the United States, or about 2 per cent of the population, suffering from some sort of heart trouble. Dublin estimates that fully 25 per cent of all heart disease comes from acute rheumatic fever and that the most common form of heart trouble is associated with hardening of the arteries. Forty per cent of all cases are of this type. Both rheumatism and hardening of the arteries are quite generally conceded to be caused by focal infection of some form. Hence, it is conservative to charge fully one-half of the death and invalidism which results from heart trouble in the ultimate analysis, to the pyogenic bacteria.

In addition to this, approximately one hundred thousand die annually in the United States from kidney disorders, fifteen thousand from appendicitis, six thousand from puerperal septicemia, and nearly three thousand from erysipelas. Some of these afflictions are due entirely to the pyogenic cocci, others only in part, but it is evident from the list that these organisms outrank such plagues as pneumonia and tuberculosis as destroyers of man.

Prevention.—The pyogenic cocci are "opportunists" and usually enter the body of their host only after the natural defenses are reduced. It is evident that the greatest security against their attack results from maintaining the body in its highest state of efficiency. Injuries to the skin and mucous membranes should receive proper attention. Over-heating, exposure to cold, fatigue, and excesses in general which may render the individual more susceptible to respiratory diseases should be carefully guarded against. Measles, influenza, and other communicable diseases render the body more susceptible to invasion by pyogenic

cocci. The prevention of these diseases not only reduces the mortality from this cause but also prevents many sequels due to these secondary invaders. Common colds are often looked upon as of small importance, but probably in a majority of cases they are the direct cause of the infected sinus or throat which later become permanent foci of infection contributing to the many diseases considered above. A common cold is not only an inconvenience lasting a day or so, but it is a potential danger which may in time be the cause of permanent injury, invalidism, or even death. Hence, the avoidance of colds is a great preventive measure, and the key-note of avoidance rests on remembering that they are communicable and are conveyed the same as other communicable diseases.

Recent discoveries in nutrition offer excellent suggestive material. Diets low in vitamin B apparently render animals more susceptible to sinus infection, and it is quite well-established that proper diets containing sufficient calcium salts and an appropriate amount of vitamin D, if given early in the life of the individual, will protect against teeth troubles and thus prevent much focal infection.

The advice of Billings is sound: "As far as possible, as individuals and collectively, physicians should exert an influence to promote cleanliness of mind and body and thus lessen the incidence of focal and systemic infection. The encouragement of personal cleanliness and especially the care of the skin and its appendages, and the mouth and throat, should be a duty of the family physician. The necessity of cleansing the mouth, teeth and throat of all particles of food after eating should be taught as a prevention of focal infection, decay of teeth and general disease."

The findings concerning focal infection should add a new incentive for the care of the teeth, the prevention of colds, sore throat, catarrh, the proper care of gatherings in the ear and nose, and especially the maintenance of proper hygiene and nutrition. It should always be borne in mind that the best time to be concerned about focal infection is before it occurs, not after.

CHAPTER XXXV

PNEUMONIA

The part of the body attacked and the nature of the combat varies with the microörganism. Each germ has its favorite point of attack: measles, scarlet fever, and smallpox attack the skin; dysentery, typhoid, and cholera, the bowels; bronchitis, influenza, tuberculosis, and pneumonia, the lungs. The deadliest of all diseases are those which attack the lungs. Headed by pneumonia and followed by tuberculosis, influenza, and bronchitis, these diseases account for from one-fourth to one-third of the annual death rate.

There is nothing slow and insidious about pneumonia. One moment its victim is well and strong; the next there is a stabbing pain in the chest, a teeth-chattering chill, followed by a high fever, and a flushed face. There is a cough, a rusty, blood-stained expectoration, and the victim is in the midst of a life and death struggle. The combat is short, sharp, and decisive. If the patient is strong enough to get the best of his assailant and is among the fortunate three-fourths, the assailant suddenly gives up and departs just as abruptly as he came. The triumph over the arch enemy is just as wonderful as the bursting forth of the sun after a hurricane, but like the hurricane it leaves the earth strewn with its victims.

Historical.—Pneumonia was known and recognized as a highly fatal disease of the lungs by the father of medicine, Hippocrates. He associated it with conditions of the weather and taught that cold northeasterly winds brought on disorders of the breast, sides, and lungs. Apparently the contagious nature of the disease was not recognized until the beginning of the sixteenth century. Since then numerous epidemics of the disease have been noted. For centuries it has been observed that it is more prevalent and more highly fatal under crowded conditions, such as occur in prisons, mining camps, and armies. The pneumococcus was first seen by Sternberg, late Surgeon General of U. S. Army in 1880. He found it in his own saliva and learned that when it was injected into rabbits their blood soon teemed with

the microörganism. The same year Pasteur independently saw and described the germ, but it was not until 1884 that Fränkel made a minute study of the organism and suggested it as the cause of pneumonia. Since that time it has been shown that pneumonia may be caused by a number of bacteria which may find their way into the lung tissue, but the principal offender is the pneumococcus.

Extent of the Disease.—As the cause of death, pneumonia is first on the list of infectious diseases and second only to heart trouble in the number of deaths it causes. During 1925 it caused over 96,000 deaths in the United States alone. It averaged a victim every five minutes night and day throughout

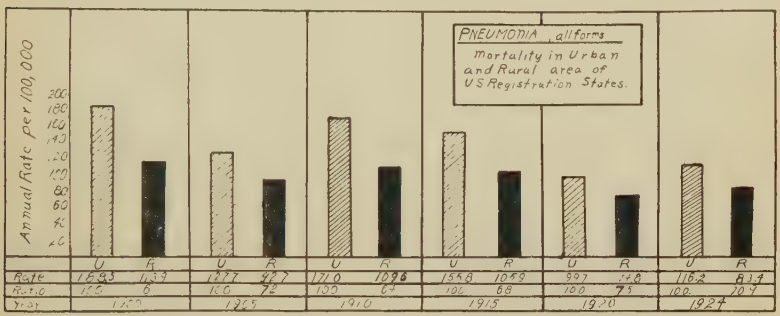

PNEUMONIA, all forms
Mortality in Urban and Rural area of U.S. Registration States.
Annual Rate per 100,000
U R U R U R U R U R U R
1910 1915 1920 1924

FIG. 103.—Pneumonia in city and rural districts.

the year. It caused more than three times the number of deaths caused by typhoid, malaria, smallpox, measles, scarlet fever, whooping cough, and diphtheria combined. However, the most depressing point of all is that apparently pneumonia is on the increase. This is due to a number of factors: (1) Crowding, which favors spread; (2) the devitalizing effect of present day life; (3) the greater number of individuals who live to the age when susceptibility to the disease increases. The death rate from pneumonia per 100,000 in 1910 for individuals over 60 years of age in the United States was 734. (4) Modern methods have made the diagnosis of the disease more accurate; hence, more cases are recognized than was formerly the case. This would cause an apparent increase.

Pneumonia is considerably more prevalent in the city than in the country, as may be seen from Fig. 103. Whenever great numbers of people are brought together the opportunity for

disease spreading is increased. Out of the 4,000,000 men in the army during the World War, 40,000 perished in combat, and about 47,000 died from disease. Pneumonia caused more than 50 per cent of the deaths prior to the influenza pandemic in 1918 and 93.7 per cent during the period covered by the pandemic. Excluding the influenza period from consideration, pneumonia was nine times more frequent among the men in the army than among civilians of the same age. Pneumonia attacks all ages, but it is more prevalent among two extremes—the child under six and the adult over sixty. "Under ordinary conditions the pneumonias reap their richest harvest at the two extremes of life. During the first year there is only one other disease, infantile diarrhea, which is more fatal. Among the aged and those suffering from chronic diseases pneumonia is the kindly friend who, having prepared the bed for the body worn out with the work and worry of life's short day, administers the soporific which induces the sleep of the eternal night. On the whole, the old man rowed across the Styx by pneumonia is not compelled to pay a heavy toll in pain." Hence, Osler states: "Pneumonia may well be called the friend of the aged. Taken off by it in an acute, short, not often painful illness, the old escape those 'cold gradations of decay' that make the last stage of all so distressing."

Pneumonia occurs in all climates but shows geographical differences. It is said to be more fatal in the United States than in England. According to some authorities this is due to the dry, over-heated air of our homes, offices, and work rooms. It is more prevalent in the winter and spring months. This is not directly due to the cold, but probably to other predisposing factors such as influenza and common colds, and especially to the over-crowding which occurs at these seasons.

Types of Pneumonia.—Pneumonia occurs in two recognized forms: (1) The lobar, fibrinous, or croupous pneumonia; and (2) lobular, or broncho-pneumonia. In lobar pneumonia one or more lobes of the lungs are involved in an inflammatory process. In this type there is practically always one group of bacteria found in the lungs and frequently in the blood, the pneumococci. It is a disease with well-defined, uniform onset, usually with an initial chill, rapid elevation of temperature, pain in the side, cough, and bloody expectoration. In favorable cases it runs a fever of about seven days and terminates abruptly. Broncho-pneumonia is caused by a variety of microörganisms. The pneumococcus is the most common single cause, but many

others which are common inhabitants of the mouth may give rise to the disease. In this type the bronchi, or small air passages, and even the smallest ramifications in the lungs together with the adjacent or terminal air vesicles, and the neighboring lung are the site of the inflammation. It does not present the clear-cut symptoms of the lobar type but occurs in various modifications, depending upon the extent of the lung involvent. This is the type that is most common in children.

Causes.—In pneumonia one can distinguish the immediate cause, the germs, and the predisposing or banal causes. It is not well understood how the predisposing causes act, but they in some way lower vitality so that the germ can enter. The relationship of age, season, city life, and geographical distribution has already been considered.

It has long been recognized that chronic drinkers are prone to contract pneumonia, and the outlook for such individual when they do contract the disease is grave. Hunger, fatigue, wet, and cold all appear to be predisposing causes. It is generally recognized that a patient suffering with any of the debilitating diseases such as diabetes, nephritis, typhoid, and measles is more likely to contract pneumonia than is a healthy individual. But, after all is said, it must be remembered that the direct cause is the specific microörganism which, in the case of lobar pneumonia, is nearly always the pneumococcus, as may be seen from the following results obtained at the Rockefeller Institute and extending over several years:

MICROÖRGANISM ASSOCIATED WITH LOBAR PNEUMONIA

	No. of cases
Pneumococcus	754
Streptococcus pyogenes	7
Influenza	6
Mixed infection	6
Staphylococcus aureus	3
Friedländer's bacillus	3
Streptococcus mucosus	1

The Bacterial Cause of Pneumonia.—The pneumococcus is usually the immediate cause of lobar pneumonia and is the most common single cause of broncho-pneumonia. It is a small micrococcus known by a number of names—diplococcus pneumonia, micrococcus lanceolatus, the Fränkel's pneumococcus, or more briefly, the pneumococcus. It occurs most commonly in

pairs, each organism being roughly oval with one extremity oval and the other pointed. This gives to it a resemblance of the flame of a candle, or the head of a lance. Usually the rounded ends of the two cocci are adjacent. At times, especially when grown on artificial media, it may occur as chains. Its longest diameter is about 1 micron.

A well-defined capsule usually surrounds the pneumococcus. This is especially true when the organism is taken direct from body exudates, or when grown in body fluids such as milk and blood. Those cases which have high mortality usually are caused

Fig. 104.—Pneumococcus Type I. (*From Ford, A Text-book of Bacteriology.*)

by definitely capsulated organisms. These observations have led to the conclusion that the capsule has a protective influence and shields the organisms against the body tissues. The organism does not produce spores, is non-motile, and stains readily with the aniline dyes.

Types of Pneumococci.—Biological tests have shown that different races of the pneumococci occur. It is found that if a horse is repeatedly inoculated with definite strains of the pneumococcus the animal's resistance is increased, and eventually the serum of its blood when injected into white mice protects them against otherwise fatal doses of this particular strain of the pneumococcus but will not protect against other strains. Fur-

thermore, it is also found that such a serum had the power of agglutinating the strain which had been used but no others. By such tests it has been found that the pneumococci can be grouped into types which are indicated by the Roman numerals I, II, III, IV. The relative frequency of the occurrence of the various types together with the mortality of each is given below:

Type of pneumococci	Relative incidence	Mortality percentage
I	33.3	25–30
II	33.5	25–30
III	13.0	50–60
IV	20.3	Usually a low mortality

The importance of this grouping rests in the fact that for some types there is a quite effective serum. For instance, in Type I the mortality is normally from 25–30 per cent of the individuals contracting the disease. However, when the serum is given the mortality is reduced to from 6–8 per cent. The bacteria comprising Types III and IV are morphologically and culturally different from each other as well as from Types I and II, but Types I and II are indistinguishable from each other except by the agglutination reaction. Hence, when a patient is suffering from pneumonia the organisms are typed to determine whether the serum should be given. The typing is made as follows: A bit of the sputum is well washed, emulsified in a salt solution, and injected into the peritoneal cavity of a mouse. In from 5 to 8 hours the peritoneum contains a strong and fairly pure growth of pneumococci. This is washed out with salt solution and centrifuged. The bacteria are then suspended in a salt solution and tested against serum from horses which have been treated with known types. At other times the culture for the agglutination test is obtained by inoculating the washed sputum into broth containing glucose and defibrinated rabbit blood. After from 5 to 6 hours the resulting growth is tested with the various type-agglutinating sera. Good results have followed the use of the serum in Type I, encouraging results with Type II, and results of doubtful value in Type III. No serum has been prepared for Type IV, as it is composed of a great variety of organisms.

Distribution and Sources of Infection.—Pneumococci are apparently normal inhabitants of the human mouth and throat, for bacteriological examinations have shown them to be present

in the mouth and throat of over one-half of the individuals examined. This fact at first led to the belief that the healthy human carrier was the source of pneumonia. After it became possible to type the pneumococci, it was found, however, that most pneumococci found in the mouth of healthy individuals belong to Type IV. Types I and II seldom, if ever, are found in the mouth and throat except in individuals suffering with the disease, recently convalescent from it, or those who have been in contact with active cases of the disease. Hence, in so far as Types I and II are concerned the sources of infection are the discharges of the nose and throat of the ill, of the recently convalescent, and of those who have been in contact with active cases. There is some evidence that the organisms may survive for a short time in dust; hence, rooms recently occupied by a pneumonia patient may be a source of danger.

Most of the pneumococci found in the mouth of the healthy carriers belong to Type IV, with possibly a few of Type III. The healthy carrier is of importance in these types of pneumonia, but even here the danger is greatly mitigated by the fact that Type IV has a very low mortality. Furthermore, those carried by the healthy person are likely to be of low virulence and invade only after the resistance is broken down by disease, cold, fatigue, hunger, and so on. Hence, it can be concluded that pneumonia is spread by direct contact with infected persons or articles freshly soiled with the discharges from the nose or throat of infected persons, and possibly to a very limited extent by dust of rooms occupied by infected persons.

Properties of the Pneumococci.—Some pneumococci grow on the ordinary culture media but never luxuriantly. In fact, they usually die in less than a week on such media if kept at body temperature. They usually retain their vitality considerably longer in the ice chest, especially if light and air are excluded. They must be transferred frequently in order to keep them alive, and it is customary at intervals to pass them through the body of a mouse in order to maintain their virulence. Their temperature range is narrow, growth taking place as a rule between 25° and 42° C. They are killed by heating to 52° C. for ten minutes and are quickly destroyed by the ordinary disinfectants. They may live and maintain their virulence for months in dried sputum and withstand low temperatures very well. All pneumococci are soluble in animal bile. This is used largely to differentiate them from the streptococci.

Pathogenicity.—The susceptibility of different species of animals to the pneumococci varies widely. Mice and rabbits are very susceptible, whereas chickens and pigeons are immune. All clinical evidence points to the conclusion that man's natural immunity to the pneumococci is comparatively high, probably midway between the two groups cited. A factor of the greatest importance is the variation in the virulence. Pneumococci may be rendered extremely virulent for mice by animal passage, but on cultivation on artificial media they lose this property just as rapidly. As a general rule, Types I and II tend to maintain their virulence better than those of Type III. It is probable that there is a wide variation in the immunity of human beings toward the various strains of pneumococci. Yet it is remarkable how uniform the susceptibility is among experimental animals. For instance, it is found that if the minimum lethal dose of a number of cultures for a number of mice be determined, it is found that practically all mice can be infected with this dose.

Immunity.—Second attacks are more common in pneumonia than in any of the other acute diseases. This has led some to conclude that an attack of pneumonia produces no lasting immunity. One attack often appears to predispose to subsequent attacks. However, it is evident that there must be a short immunity; otherwise, recovery would be less frequent. Vaughan, in commenting on the greater prevalence of pneumonia among the soldiers from the rural districts as compared with those of the city, recognizes an acquired immunity, when he states: "The figures which we present convince us that the greater susceptibility to pneumonia of the soldier from the rural district as compared with his comrade from the urban home is due to the fact that the former has not acquired the degree of resistance to this disease possessed by the latter. Findings such as these, together with many others, have caused some authorities to conclude that there is an acquired immunity in pneumonia, but that it is specific and that second, third, and subsequent attacks of pneumonia come from different races or types. The clinical evidence so far obtained, therefore, indicates that following an attack of pneumonia due to pneumococci of Types I or II there may be present a considerable grade of immunity against infection with the respective types of pneumococci. Following pneumonia due to pneumococci of Type III, on the other hand, the evidence is inconclusive that the attack is followed by any great or prolonged immunity against these organisms. So far as

pneumonia due to pneumococci of Type IV is concerned, there is no clinical evidence that an attack of pneumonia due to an organism of this group is followed by an immunity against other strains of this group or against pneumococci of the other types."

How to Prevent Pneumonia.—The first and most important thing in the prevention of pneumonia is the avoidance of the germ. Pneumonia is a communicable disease and is spread directly from individual to individual. Unnecessary contact with those ill with, or recently convalescent from, pneumonia should be avoided. If one's duties are in the sick room care should be taken to cleanse the hands thoroughly, especially before eating. The sputum of the patient should be collected and burned. All utensils which come in contact with the patient should be disinfected and kept solely for his use. Food coming from the sick room should be burned. Bedding and personal clothing of the patient should be boiled.

The pneumococci multiply only in the human individual and are spread by direct contact. Special precautions should, thus, be taken to prevent the spread of the secretions of the nose and throat. Foreign objects, including the fingers, should be kept out of the nose and mouth. Promiscuous spitting, careless sneezing and coughing should be avoided, as they tend to spread pneumonia as well as colds and influenza. There are carriers of the disease, whose immunity is high enough to keep in check the organism, but there is no assurance that this will be the case when this same organism is transferred to the mouth of a second individual.

One should shun crowds in so far as practicable. This is especially true during epidemics, for normal individuals are carrying low virulent pneumococci, but during epidemics the virulent strain is often, also, present. The evidence is conclusive that over-crowding is a very important factor in the spread of the disease. It is especially important that crowds be avoided when the respiratory passage of a person is inflamed by a cold. A catarrhal condition predisposes to pneumonia, and it is safe to say that in every crowd there is a carrier of at least some type of the germ.

Undue exposure to wet and cold should be avoided, and the air of the living, working, and sleeping rooms should be clean, cool, and of the proper humidity. Excessive heat is injurious. Anything that causes a congestion of the mucous membranes of the lungs gives the pneumococci a chance to develop.

Diseases such as typhoid, measles, and diabetes render one more susceptible to pneumonia, consequently, these diseases should be prevented, for they directly endanger life and also are predisposing to pneumonia. Most of the deaths which occur in measles are due directly to pneumonia.

The general health should be guarded. One should receive proper food, sufficient sleep, and a reasonable amount of physical exercise taken regularly and systematically; worry, over-work, over-eating, unnecessary exposure, and undue fatigue should be avoided. It is at least possible that more persons are succumbing each year to pneumonia, because life is becoming increasingly difficult. "We hurry too much, we worry too much, we play too little. Our lives are top-heavy, and the equilibrium is unstable. This is obvious in theory but we do not act upon it. Most of us get our fresh air through a distant, narrowly-opened window, our recreation at the movies, our physical exercise in walking two blocks to the cars."

However, it would be wrong for one to conclude that physical fitness alone protects against pneumonia, for, "among the robust and vigorous of all ages pneumonia is the cowardly assassin, who, watching the every ebb in the flow of life's forces, strikes at a vital part at the most opportune moment. It has long been a matter of observation that epidemics are most fatal among the most virile. An old writer, discussing typhus fever in England and Ireland said that death went abroad among the community, picking out the lustiest and handsomest, even as you or I should go through a flock of sheep. The Typhoid Commission in 1898 found that more than 90 per cent of the men who developed typhoid fever had no preceding intestinal disorders. In the World War in the midst of the great epidemics of influenza and pneumonia, it was a common observation that the most robust were most severely stricken and supplied the largest percentage of fatalities." This is due, in a measure, to their robust condition carrying them into the danger and keeping them about when they should be receiving hospital treatment. But this is not the only factor, for Vaughan argues "that the infectious diseases owe their lesions and symptoms to the destruction of the invading microörganism by secretions from the body cells. The strong and vigorous man pours out these specific secretions in such abundance that enough bacterial cells are split within a short time to set free a sufficient amount of poison to lead to a fatal result. Nature over-reaches herself in the combat. Nothing

worse could happen to a man with typhoid fever than a sudden disruption and destruction of all the bacterial cells in his body. Certainly, this would be true if such disruption should occur after the invading cells had accumulated in sufficient numbers to furnish a fatal dose of the protein poison on their disruption. In epidemics the body cells of the strong and vigorous man go into action with energy, and such a man either recovers promptly, or is more likely to die than his less robust comrade whose body cells destroy less abundantly and less rapidly the invading organism. Epidemic diseases certainly do not improve the race by killing off the unfit, but like war they destroy the flowers of the race, and in so doing rob the future generations of the inheritance of certain virtues. Victory in combat with epidemic diseases is not always to the strong."

CHAPTER XXXVI

TUBERCULOSIS

The events which burn themselves into the human mind are the tragedies, the quick and sudden changes, the catastrophies of sea and land. Survivors long remember and relate the fury of the hurricane, the sorrows of the shipwreck, the suffering from the earthquake, the want and misery from a great conflagration,

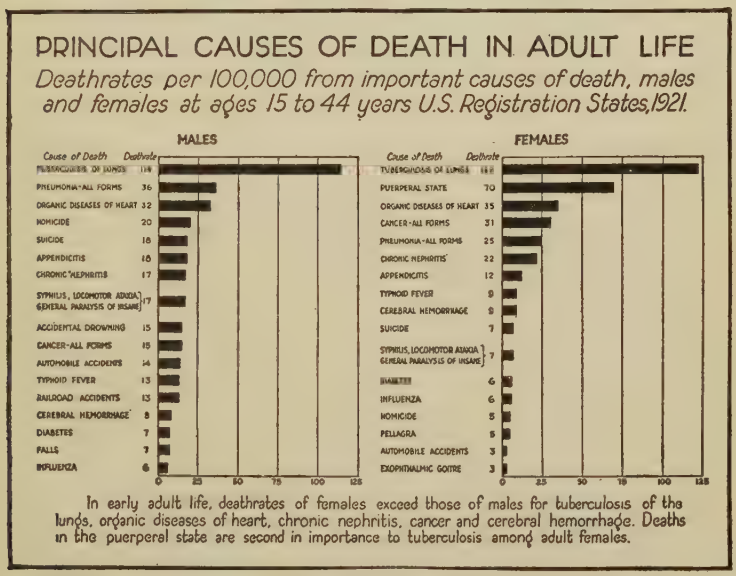

PRINCIPAL CAUSES OF DEATH IN ADULT LIFE
Deathrates per 100,000 from important causes of death, males and females at ages 15 to 44 years U.S. Registration States, 1921.
MALES
Cause of Death
Deathrate
TUBERCULOSIS OF LUNGS 114
PNEUMONIA-ALL FORMS 36
ORGANIC DISEASES OF HEART 32
HOMICIDE 20
SUICIDE 18
APPENDICITIS 18
CHRONIC NEPHRITIS 17
SYPHILIS, LOCOMOTOR ATAXIA, GENERAL PARALYSIS OF INSANE 17
ACCIDENTAL DROWNING 15
CANCER-ALL FORMS 15
AUTOMOBILE ACCIDENTS 14
TYPHOID FEVER 13
RAILROAD ACCIDENTS 13
CEREBRAL HEMORRHAGE 8
DIABETES 7
FALLS 7
INFLUENZA 6
0 25 50 75 100 125
FEMALES
Cause of Death
Deathrate
TUBERCULOSIS OF LUNGS 118
PUERPERAL STATE 70
ORGANIC DISEASES OF HEART 35
CANCER-ALL FORMS 31
PNEUMONIA-ALL FORMS 25
CHRONIC NEPHRITIS 22
APPENDICITIS 12
TYPHOID FEVER 9
CEREBRAL HEMORRHAGE 9
SUICIDE 7
SYPHILIS, LOCOMOTOR ATAXIA, GENERAL PARALYSIS OF INSANE 7
DIABETES 6
INFLUENZA 6
HOMICIDE 5
PELLAGRA 5
AUTOMOBILE ACCIDENTS 3
EXOPHTHALMIC GOITRE 3
0 25 50 75 100 125
In early adult life, deathrates of females exceed those of males for tuberculosis of the lungs, organic diseases of heart, chronic nephritis, cancer and cerebral hemorrhage. Deaths in the puerperal state are second in importance to tuberculosis among adult females.

Fig. 105.—Principal causes of death in adult life. (U. S. 1921.) (*After Dublin.*)

and the terrible loss of life from a swift acting plague, or war; but the slow and relentless foes of mankind are noted, commented upon, and taken as a matter of fact. Two of man's greatest microbial destroyers are the pneumococci and tubercle bacilli. They take greater tolls of human life in peace and war, on land and sea, than the more spectacular tragedies which com-

mand our attention. They have in common a special preference for the respiratory system, but their injury is not alone confined to the lungs. Each may invade and destroy nearly any tissue of the body. They are alike in that they invade the body of man best when general vitality has been reduced by chemical, physical, or biological means. As pointed out in the preceding chapter the pneumococci strike quickly and without previous warning. The tubercle bacilli are slow and insidious. The combat between man and the pneumococci is short, sharp, and decisive. Even when man is victorious he is weakened for the next struggle, for the resulting immunity is low and short-lifed. The struggle with the tubercle bacilli is long, for the powers of the combatants are usually nicely balanced, and it often results in a draw; but when man is once triumphant, there is a long and lasting immunity. The victims of pneumonia are probably on the increase, whereas those of tuberculosis have shown a steady and consistent decline during the past half century.

History.—Tuberculosis probably claimed its victims from among the lower animals long before the advent of man. There is evidence gleaned from the unmistakable scars on ancient Egyptian mummies, inscriptions on old Babylonian tablets, and early Greek manuscripts that it worked havoc even among the early human races. The Veda of India, the Zend-Avesta of the Parsees, and the writings of Hippocrates all abound in reference to it. Hippocrates left definite evidence that he recognized it as a disease of the lungs, and one of his contemporaries considered it to be contagious. Galen taught that "it is dangerous to live with consumptives and with those whose foul breath imparts a heavy odor to the room in which they live."

Closely interwoven with these careful observations of the masters are the many fanciful theories of the other classes of people. Some taught that the disease was caused by a vicious disposition of the body juices; others, that it was due to a strange ferment; and still others, that it was caused by a sharp corroding humor falling from the brain upon the lungs. Throughout the Middle Ages, the light of science was extinguished, and it was not again until the seventeenth century that intelligent discussion of the nature of tuberculosis appeared. The term "tubercle" was employed by the anatomists of the seventeenth century to refer to the nodules of various sizes in diverse tissue, and about the middle of the century Sylvius applied the term to the nodules

in the lungs of consumptives. It remained, however, for Laennac (1781–1826) to lay the real foundation for our knowledge. He recognized the different forms of the disease, and developed the method of auscultation by which he was able to detect the disease in the living subject.

Villemin (1827–1892) used the experimental method and proved that tuberculosis is transmissible from animal to animal. He inoculated numerous and various animals in diverse ways with sputum and other products from tubercular men and cattle, and found that tuberculosis resulted. But even at this late date many questioned his methods and conclusions. Pidoux contended: "Experiments on animals give such and such results and you, instead of controlling them by clinical experiments and by all the accepted facts of human physiology, construct upon them a general theory of human tuberculosis and all diseases! For it you upset all the ideas already acquired. We must accept over night that phthisis falls from the clouds and that in its pathogenesis, the subject himself, habitus, hygienic surroundings, hereditary and diathesis are of no account, that all depends upon an impossible tuberculosis virus originating without doubt in a tuberculous individual who had it from some other individual, and so on to the first man, who, however, had it from nobody at all and must have created it himself out of nothing."

It remained for the glorious work of the immortal Robert Koch to reveal to the eye of man the "impossible tuberculous virus" in 1882. He not only showed that the organism is in the sputum of tuberculous patients and lesions in the various parts of the body, but he also succeeded in growing it in artificial media and demonstrating its pathogenicity. Since that day many patient workers in various lands have added to this knowledge, until today we possess several outstanding facts concerning tuberculosis. (1) It is an accepted truism that without the specific organism there can be no tuberculosis. (2) Children are born free of the disease. (3) Tuberculosis infection increases with age and when adult life is reached between 50 and 100 per cent of the population in civilized countries show signs of the infection. (4) The majority of civilized individuals get the infection, but only about one in ten develops the active disease. (5) Tuberculosis is both a preventable and a curable disease.

Prevalence and Cost.—We look back on the four years of our civil war with its fatality of two hundred and fifty thousand killed and think what a loss it was to the nation, yet during the past

three years more than this number met death in the United States from a foe which claims more victims than does war. It has been estimated that during the nineteenth century fourteen million lost their lives on the battlefield. During the same period over thirty million died of tuberculosis. The twentieth century has been ushered in with gigantic wars, yet it is a safe guess to say that during this century more will die from the "Great White Plague" than on the battlefield.

In 1925 approximately 90,000 persons died in the United States from tuberculosis, that is, nearly 8 per cent of the deaths were due to this disease. In some foreign countries the rate is still higher. It claims yearly over three times the victims taken by scarlet fever, measles, typhoid, diphtheria, and whooping cough combined. It is exceeded among the communicable diseases only by pneumonia. Pneumonia claims the greatest numbers at the two extremes of life, but tuberculosis takes man just when life should mean the most to him. It has been estimated that 30 per cent of all deaths between the ages of fifteen and sixty are caused by it.

The number ill with this disease has been variously estimated at from five to twenty times the number that actually die of it. It is probably conservative to place the morbidity rate at ten times the death rate; this would mean that there are approximately 1,000,000 cases in the country, or about one per cent of the population ill with the disease. The number infected is much higher. Over 90 per cent of city dwellers show signs of tuberculosis in their bodies after death; 60 per cent show signs in the lungs. The tuberculin test which is used in living patients is so delicate that it not only reveals active and serious tuberculosis, but also the presence of minute and even practically healed small, diseased areas, in any part of the body. This test shows 90 per cent of all 12-year-old children and 60 per cent of the young healthy adults to be tubercular. When the whole population of city and country are considered, it is estimated that 50 to 70 per cent have been infected. Hence, while one in every twelve deaths is due to tuberculosis, eight in every twelve individuals become infected with the disease. In most cases, however, the infection is so slight that the patient is never conscious of it.

The mortality from tuberculosis varies widely with a number of factors. There is a greater mortality among the negro population than the white. More males than females die of the disease probably because there is a greater opportunity for infection. It

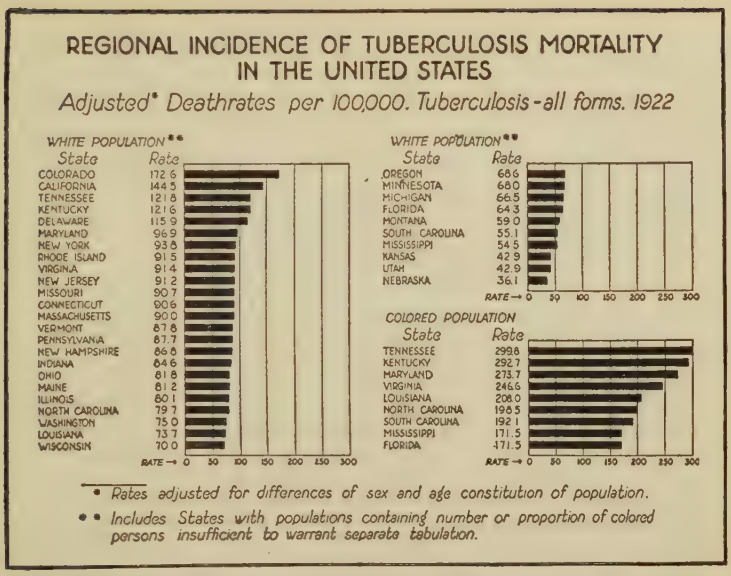


FIG. 106.—Regional incidence of tuberculosis mortality in the United States (*After Dublin.*)

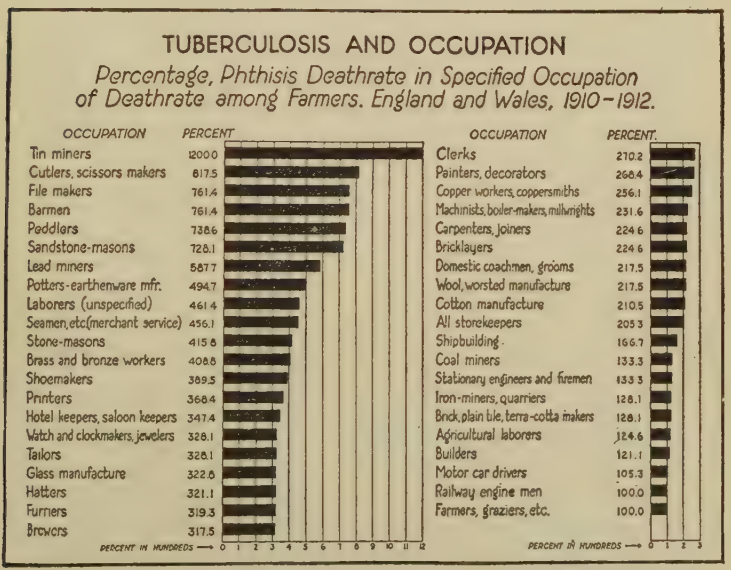


FIG. 107.—Tuberculosis and occupation. (*After Dublin.*)

is more prevalent in the city than in the country districts and in the southern than in the northern and western parts of the country. The mortality is lowest in Nebraska, Utah, and Kansas; and highest in Colorado, California, and Tennessee. It must not, however, be concluded that the climate of these latter states is conducive to the contraction of tuberculosis, for many that die there are tuberculous patients who have flocked to these regions with the hope of regaining their health.

There is also a relationship between the tuberculosis death rate and the occupation. Statistics show miners, cutlers, scissors- and file-makers leading the list, whereas the mortality among farmers, railway engineers, and motor car drivers is low. Two factors enter: (1) the opportunity for infection which undoubtedly is greater in some occupations than in others; and (2) the conditions which are more conducive to ill health in some occupations than in others. Hazardous occupations are those in which the person is constantly inhaling sharp or irritating particles which cause small lesions in the respiratory epithelium and admit the bacilli.

During the past twenty years there has been a gradual but consistent decline in the death rate from tuberculosis. In 1900 it was 195.2 per hundred thousand of population in the United States for which reliable statistics are available. In 1910 the rate in the same geographical area had dropped to 164.7, or 15.6 per cent, in the 10-year period. In 1920 the rate in the same states was 112. This is 42.6 per cent less than the figures for 1900. In 1925 the rate reached the low figure of 86.8 per hundred thousand, which is considerably less than one-half of what it was in 1900. Basing his conclusion on a careful study of all the known factors, Dublin writes: "A tuberculosis rate of 50 per 100,000 will, I believe, be found to be not far from the actual figure in 1930. Please remember that in New Zealand and in Australia, a death rate from tuberculosis of 50 has already been attained. Three states of the United States in 1921 had rates below 50; in fact, two were below forty. It is for this reason that I am encouraged to consider this estimate entirely reasonable. It may even be lower, provided an effort is made during the next years to apply the lessons of our experience with this disease during the last twenty or thirty years. It may be much lower also if some effective biological or other new method is discovered for the treatment or prevention of tuberculosis. The tuberculosis movement must not rest on its laurels nor be allowed to drift

along easy ways. The disease is on the decline. This is an opportune time to apply even greater energy along approved lines, that there may be no diminishing but rather cumulative returns in life saving."

This general decline is attributed to different factors by various workers. The first explanation which is espoused by most workers in the field of public health is that the decline in the tuberculosis death rate is due to the great improvement in the general well-

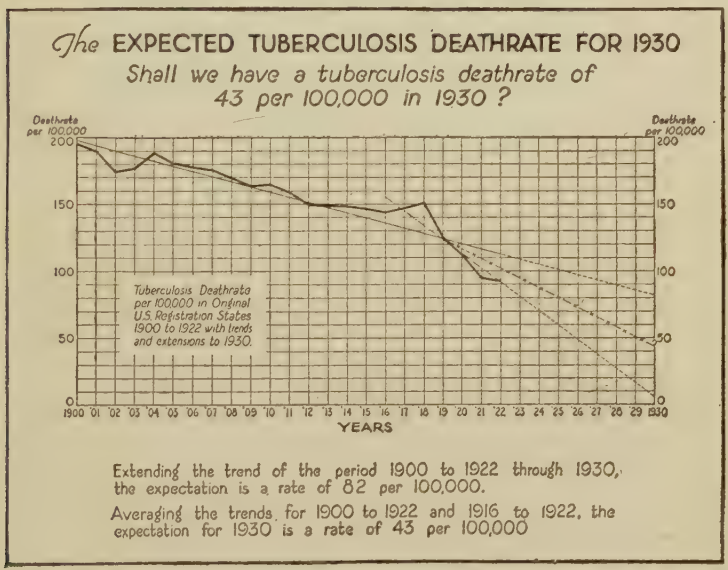

The EXPECTED TUBERCULOSIS DEATHRATE FOR 1930
Shall we have a tuberculosis deathrate of 43 per 100,000 in 1930 ?
Deathrate per 100,000
200
150
100
50
0
Tuberculosis Deathrate per 100,000 in Original U.S. Registration States 1900 to 1922 with trends and extensions to 1930.
1900 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 1930
YEARS
Extending the trend of the period 1900 to 1922 through 1930, the expectation is a rate of 82 per 100,000.
Averaging the trends for 1900 to 1922 and 1916 to 1922, the expectation for 1930 is a rate of 43 per 100,000

FIG. 108.—Decline in the tuberculosis death rate and the expected death rate for 1930. (*After Dublin.*)

being of the population. Those who hold this view say that the great masses of the population are pretty generally exposed to infection, but those who break down with the disease are the ones who lack the immunity to localize it in the very early stages. This power of immunity is being increased by the good hygienic condition which people are learning to live.

The second explanation minimizes the importance of hygiene and stresses the constitutional factor. The people holding this opinion point to the fact that the decline in death rate antedates even the knowledge of the specific organism. They consider

that it is inherent within the race. There are being produced individuals who are more resistant to the germ.

Besides the loss in life the economic loss from the disease is appalling. It is impossible to accurately evaluate this, but it has been estimated that the yearly loss to this country from tuberculosis exceeds in value that of the wheat and corn crop, and that the loss of an annual hay, milk, butter, and wheat crop would only slightly exceed the annual loss due to tuberculosis. The disease costs approximately half a billion dollars annually in the United States more than the army, nearly twice as much as the navy, and second only to the national debt in comparison with government expenditures.

Heredity.—The frequency with which tuberculosis occurs in some families early led to the idea that the disease was inherited, but when it was discovered that the disease is due to a germ which invades and injures it did not appear probable that it could be transmitted from parent to offspring in the germ-plasm. This conclusion is borne out by extensive clinical and experimental work. There are very few cases on record in which the child is born with the disease. Consequently, it is generally accepted today that the child even of tubercular parents is born free of the disease. In other words, tuberculosis is not inherited.

However, there has been much discussion in recent years as to whether or not a tendency toward the disease is inherited. It is quite generally conceded that some races, such as the American Indians and negroes, succumb to tuberculosis more readily than others; but it is hard to nicely separate the hereditary tendency from the bad hygienic conditions under which the races live. Moreover, in the United States, the death rate from tuberculosis among the Jews is only one-fourth of what it is among the general population, but the number of infected is probably greater than in other races. Guerin has tried to defend the idea that blondes with auburn silky hair and pale freckled skin of fine texture are especially prone to contract tuberculosis. It is probable that certain tendencies to the disease are inherited, but even this is challenged by some who point to cases in which calves of tubercular mothers separated from their mothers at the time of birth, fed with milk from healthy cows, and shielded from infection later did not contract tuberculosis more readily than the offsprings from healthy parents. Hence, while we conclude that tuberculosis is not inherited, resistance seems to be built up in a race in which the disease has long been prevalent.

Undoubtedly the main reason children of tubercular parents are more prone to contract the disease than other children is due to the greater opportunity for infection. They are constantly exposed to infection at an age when their immunity is low. The infection may be brought about by the mother, who tastes the porridge with a spoon to assure herself that the temperature of the food is correct, who wipes the baby's eyes and mouth with her handkerchief moist with bacillus-laden sputum, or who kisses the child upon its lips. When the child becomes old enough to creep or walk it is constantly sucking its dirty fingers covered with the sputum-laden dust.

Cause of Tuberculosis.—Tuberculosis is caused by a rod-shaped organism discovered by Koch in 1882. In its absence there can be no tuberculosis. Yet only one in ten or twelve infected with the organism die of the disease. Moreover, the work of recent years has demonstrated that the disease is curable, if it is detected in the incipient stages and the patient adheres to a carefully regulated hygienic regimen. This has led to the belief that there are predisposing or banal causes in tuberculosis. Rosenau writes: "In man the balance between immunity and susceptibility to tuberculosis is delicately adjusted; there is a very small factor of safety. The resistance to infection may be increased by attention to personal hygiene, fresh air, and good food; immunity may readily be broken down by any weakening influence; herein lies the keynote of personal prophylaxis."

Irritating fumes and sharp particles when inhaled cause lesions in the epithelium of the lungs, and, open the way for the too-often present tubercle bacilli. English statistics show that if the death rate from tuberculosis among agriculturists is taken as 100, then, that of tin miners is 1,200, cutlers 817, file-makers 761, car men 761, and stone masons 728. There are a number of complicating factors entering into a consideration of such statistics. The greater prevalence of the disease in these occupations throws the health worker in constant contact with the germ. Yet even when this is considered, it can be concluded that many dusty occupations increase the hazards of tuberculosis.

Wild animals in a state of nature are not naturally liable to the disease, but when confined in zoological gardens they quickly succumb. The cattle from the mountain ranges of the west seldom suffer from tuberculosis, and farmers, engineers, and motor drivers all show a low mortality from the disease. Emery

considers exposure to a vitiated atmosphere a most potent cause of the breaking-down of immunity. "It (a vitiated atmosphere) is especially important in connection with tuberculosis, and nothing is more striking than to notice its effect on the peasantry of some regions in which in spite of exposure to abundant fresh air during the daytime, and a supply of food which certainly does not fall below the physiological minimum, and is usually more abundant, phthisis and other tubercular diseases are rife."

The beneficial influence of fresh air is due more to the general physical effect upon the surface of the body than to its effect through the lungs. Consequently, fresh air should be obtained to as great an extent as possible in the open, and not alone by the mechanical devices which are arranged to bring it into the house.

Recent research indicates that the value of outdoor life is in a measure due to the action of the ultraviolet light upon the tissue. Animals as well as plants require light rays in order to properly metabolize their food. We are all children of the sun and are directly or indirectly dependent upon it for strong, healthy bodies. The harmful effects of alcohol; the wasting diseases typhoid fever, diabetes, and whooping cough; together with fatigue, poverty, and malnutrition have long been recognized by the physicians as potent factors in the breaking-down of the natural immunity to tuberculosis.

However, one must not lose sight of the fact that even the strongest and most robust individuals in the prime of life succumb to tuberculosis, if they receive large virulent doses of the organism; consequently, it is essential to learn the source of the germs. They multiply only in the bodies of man and the lower animals and are found in close association with them. There are three kinds of tubercle bacilli—human, bovine, and avian.

Human Tubercle Bacillus.—This organism produces the disease primarily in man. It is a slender rod often slightly curved. It measures from 2 to 4 microns in length and 0.3 to 0.5 microns in breadth. In material taken from the body, sputum for example, it occurs singularly, or in pairs usually arranged at an angle, but more often lies in small heaps. In old cultures and at times from the sputum, clubbed, or filamentous forms may occur. The latter are often branched. Some observers consider these as involution forms, others consider them as normal variations of the organism. The tubercle bacilli are non-motile, possess no capsule, and do not form spores. The usual stains are

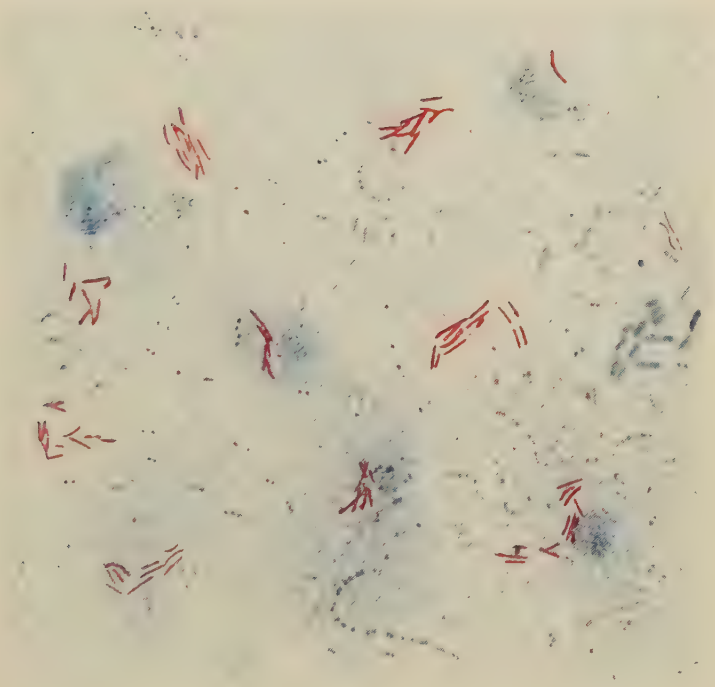


Fig. 109.—Mycobacterium tuberculosis from sputum. (*From Ford, A Text-book of Bacteriology.*)

without effect upon them unless heat is applied, or the stain allowed to act for a long time. When they are once stained, they are not easily decolorized. They may be treated with mineral acids or alcohol and still retain the stain. This has caused them to be referred to as "acid-fast," or "alcohol-fast." This property can probably be attributed to the large amount of waxy and fatty material which their bodies contain. They grow only on special media, blood serum, egg media, and the like.

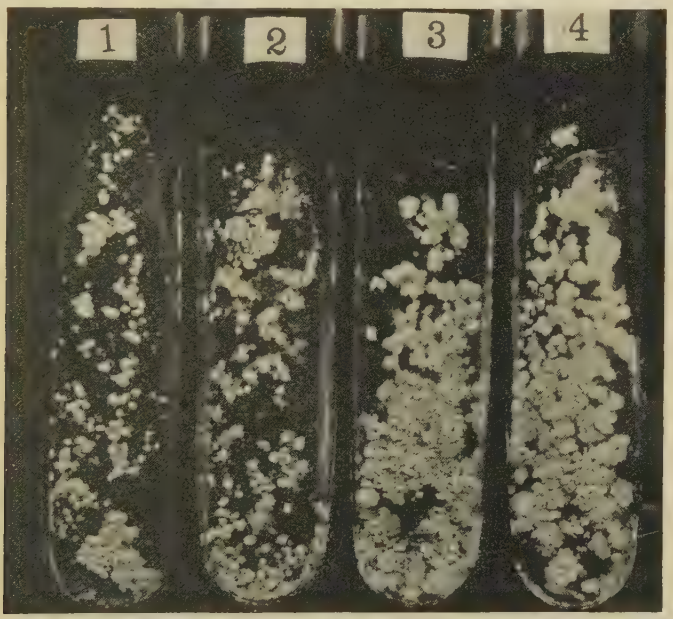


FIG. 110.—Growth of tubercle bacillus, 14 days at 370 C. The better growth in tubes 3 and 4 is due to better moisture conditions. (*After Novy and Soule.*)

After they have been grown some time on these special media, they will grow on glycerine agar. They grow best aërobically at a temperature of 37° C., are quite resistant to drying, and may survive in dry sputum in a dark, cool place for even months. Direct sunlight effects their destruction in a short time especially in the presence of oxygen. They are killed in the presence of moisture in 15 to 20 minutes at a temperature of 60° C.

They are quite resistant to dry heat, for in dried sputum they are able to withstand a temperature of 100° C. for one hour.

When free from proteins, they are readily destroyed by the ordinary disinfectants, but in sputum they are quite resistant because of the mucin of the saliva. Lysol has a solvent action on the mucin; hence it is one of the better disinfectants for this purpose.

Bovine Tubercle Bacillus.—The bovine bacillus is very pathogenic for all mammals except man. It is pathogenic for man but to a less extent than is the human bacillus. A large dose of the human bacilli injected into the calf usually results in only a local lesion, whereas the bovine type gives rise to a severe form of the disease. The distinguishing test for the two is made by rabbit inoculation. If 0.01 milligram of a bovine culture is injected intravenously, or 10 milligrams subcutaneously, into a full-grown rabbit, generalized tuberculosis results in about six weeks; whereas 10 to 100 times this amount of a human strain produces at the most a slight localized tuberculosis. The bovine bacillus is shorter and thicker than the human bacillus and grows less readily upon laboratory media. The acidity curves in glycerine broth are different.

The most important phase is the relationship of the bovine bacillus to human tuberculosis. Koch in 1901 announced that there was practically no danger of man contracting tuberculosis from cattle, but his statement was immediately challenged by many bacteriologists. Since that time statistics have been collected in many parts of the world. By 1911 Park and Krumwiede had collected a total of 1,224 observations, and from these they drew the following conclusions: (1) that children are often infected by the bovine bacillus, and the portal of entry is the alimentary canal; (2) that cervical, adenitis, and abdominal tuberculosis are the most frequent type of the infection; (3) that generalized tuberculosis due to bovine infection is less frequent; (4) that bone and joint tuberculosis is most common of the human type; (5) that the meninges are less commonly affected by the bovine than by the human bacillus; (6) that the bovine type seldom infects adults; and (7) that although cases of pulmonary tuberculosis due to the bovine type of bacillus have been reported, such cases are exceedingly rare. They estimate that about 10 per cent of all deaths caused by tuberculosis in children under five-years of age are the result of bovine infection. This is an entirely unnecessary waste of human life as the proper pasteurization of all milk would prevent nearly all cases of bovine origin.

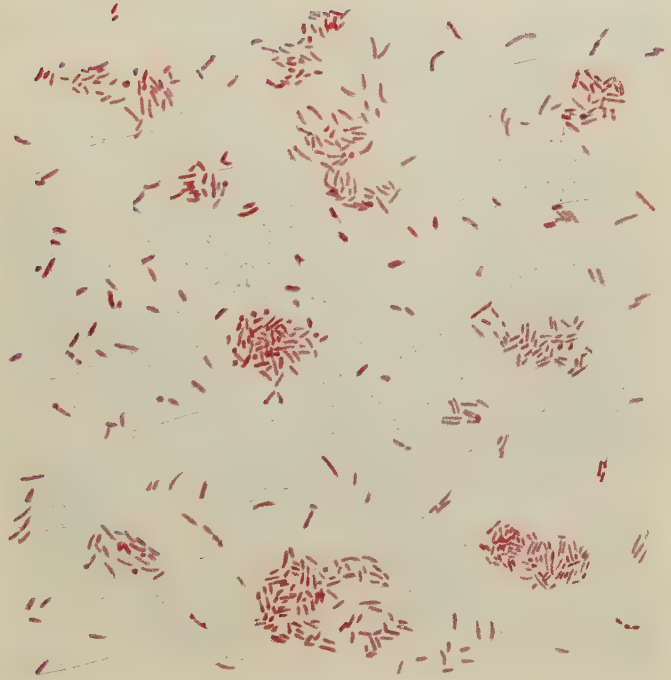


Fig. 111.—Mycobacterium tuberculosis bovine type, from glycerin-agar culture, fifteen weeks old. (*From Ford, A Text-book of Bacteriology.*)

The Avian Tubercle Bacillus.—Morphologically, the avian bacillus is like the human. It grows abundantly at the surface on glycerine media and has an optimum temperature of 40° to 43° C. It readily infects chickens, whereas the human and bovine do not. Its main importance rests on the damage it does when once it gets into a flock of chickens. A few cases have been reported in which it has been found in tubercular

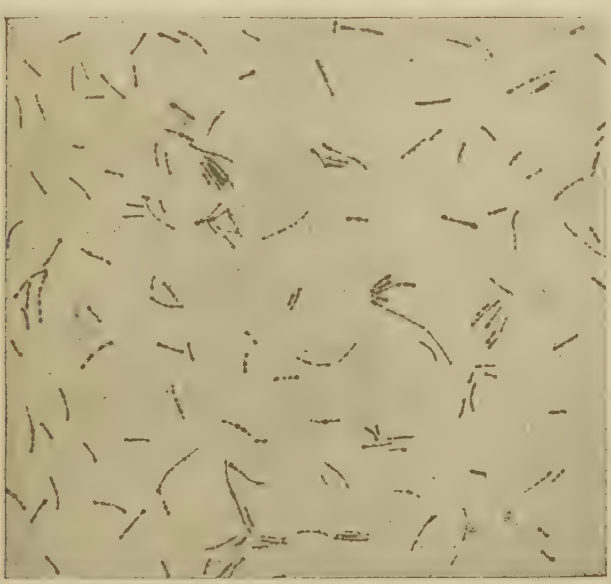


FIG. 112.—Mycobacterium tuberculosis avian type, from ten weeks culture on glycerine agar. (*From Ford, A Text-book of Bacteriology.*)

lesions of human individuals, but it must have a very low pathogenicity for man; otherwise, it would be more prevalent.

Tuberculin.—Robert Koch early in his study on tuberculosis learned that dead bacilli, or their extracts when injected into infected animals produce a different effect than when injected into healthy animals. This led him to the discovery of tuberculin which is a concentrated extract made from the tubercle bacilli. When injected subcutaneously a local inflammation results. There is also a general reaction characterized by headache, backache, and fever. Koch advocated its use as a curative agent and believed that the reactions occurred only in tuberculous subjects.

Later work showed it to have little value as a curative agent, and if not properly used might do harm. It was found that when large doses were administered both healthy and diseased animals reacted, but by nicely governing the dose it was found possible to differentiate the infected from the non-infected. Because of the extensive prevalence of infection in the human individual it has little value in human diagnostic work, but is used extensively either in its original or modified form for the diagnosis of the disease in cattle, and if properly and systematically applied it is possible to eradicate the disease in cattle.

Calmette and Wolff-Eisner found that tuberculin dropped into the eye produced a reaction. Moro rubbed it into the skin, and finally von Pirquet in 1907 found that if a very slight abrasion was made in the skin and a drop of tuberculin placed on it a local reaction occurred in tuberculous persons without causing systemic or general reactions. At first it was believed that this test had great diagnostic value, but it was soon discovered that nearly every one with the exception of very young children, healthy or tuberculous, reacted. This taken in connection with the findings at autopsy led to the conclusion that practically everyone becomes infected with tuberculosis at an early age, but that only a comparatively few became tuberculous.

Modes of Infection.—Since the tubercle bacilli do not grow in nature but in the body of man and the warm-blooded animals, these are the fountains of tubercular infection. The germs leave in the excreta of the body, sputum, feces, surface sores, and, occasionally, in the urine. Inasmuch as they may survive for months in the moist or partly dried excreta, it can readily be understood how easily they can find their way into the body of human beings. The saliva of the diseased is constantly being spread on fingers, pencils, cups, toys, and a thousand and one things which are mouthed by one and then another, to say nothing of the even more direct transfer of the organisms by the kiss.

Brown and Cummings independently have shown that forks, spoons, and cups used by people with open tuberculosis carry the bacilli. It has been further shown that when such spoons are washed, as they ordinarily are washed in the home, the wash water, rinse water, and the spoons all carry tubercle bacilli. Consequently, it is evident that great care must be used in sterilizing tableware, especially forks, spoons, and glasses used in restaurants, boarding houses, and the soft drink parlors; otherwise, they may be potent factors in the spread of disease.

The sputum of the tuberculous individuals often contains millions of the tuberculous germs, and when carelessly deposited on the street or ground may reach the fingers of playing children and later their mouths. Or the bacilli may be carried on ones shoes to the floor or rugs of the dwelling rooms where small children playing about come in direct contact with them. Then there are insects which fly or crawl directly from germ-laden sputum to the food of the home or the store. The dried sputum may be brought into the air by sweeping or by air currents and in this manner infect, for the organism may live for considerable periods in dust-laden atmosphere away from the direct rays of the sun.

There is also the infection which results from the droplets forced from the mouth during coughing. Hence, it is evident that the tuberculous individual who permits the infected droplets to fly from the unprotected mouth is endangering those in his immediate vicinity. Or, if he guards the cough with the bare hand and then shakes hands with a friend, he is making the transfer even more direct.

There may also be infection from the lower animals, and where raw milk is used there is the ever-present possibility of infection, which according to the careful investigations of Park is considerable. Infection from meat and milk can be entirely prevented by pasteurizing the milk and by inspecting and proper cooking of the meat.

Channels of Infection.—It is possible for tubercle bacilli to enter the body through either the respiratory, or digestive tracts. Occasionally, the germ enters through abrasions, and there are a very few cases on record where they have been transmitted from mother to fetus through the placenta. In the early history of the knowledge of tuberculosis, it was concluded that infection entered mainly through the respiratory tract. This opinion was held primarily for the following reasons: (1) Because the sputum of the diseased is often extremely rich in tubercle bacilli; consequently, on drying it may be ground and carried into the air. (2) Because the organism usually localizes in the lungs; therefore, tubercle bacilli carried by dust, together with infected droplets direct from the mouth of the ill, offered a plausible explanation for infection. It was, however, learned that ingested bacilli may localize in the lungs, and the point of the lungs in which they locate is similar to that occurring in natural infection, moreover, smaller numbers infect when ingested than when inhaled. These

findings directed attention to the alimentary tract as the portal of entry. Ingestion is the sole method of conveying bovine tuberculosis to man. It has been repeatedly proven that tuberculosis can be conveyed to animals by either method, and there is abundant evidence pointing to the conclusion that the same is true of the human individual. Consequently, all who have made a careful study of the subject are agreed that the disease does result from ingestion and inhalation of the germ. But which is most important? Here even careful students of the subject part company. We cannot say.

Influence of the Bacilli on the Body.—Having entered the body of the host the effect varies greatly, depending upon the number, their virulence, and the resistance of the host. Probably in the majority of cases where the number of bacilli entering are small, they are quickly destroyed and the host develops the power of handling even larger doses, or they may lodge in some tissue and cause increased growth of epithelial and interstitial cells which soon form a globular mass which walls them off from the rest of the body. Thus result minute tubercles.

Sometimes the body succeeds in killing the bacilli. At other times they only keep the microbes from growing. They remain alive and latent but ready to start their campaign of conquest anew, if the effectiveness of the barrier is reduced by infection, malnutrition, overwork, fatigue, cold, and poor hygiene.

Occasionally, these tubercular processes become chronic, the tubercle bacilli continue to grow, the infected area becomes larger and larger, and more of the organ in which they are located is destroyed. Symptoms become more pronounced because of the impaired organ, and the poisons elaborated and distributed throughout the body by the blood. In rare instances the growth may be in the walls of a venule or arteriole that eventually rupture directly into the circulation. Once in the blood stream they are carried to various parts of the body where they may locate and give rise to new tubercles. This condition is known as miliary tuberculosis.

Whenever the tubercle bacilli find conditions within the host favorable for their nourishment and growth, the tissue adjacent to the bacilli is injured; and although new cells are formed, these in time are killed, and the area of dead tissue enlarges, so that there soon results an ulcerating sore in the tissue. This process continues, the blood supply is shut off from certain tissues, nutrition stops, the cells die, and then a cavity is formed in the

living tissue. The softening area continues to necrose until it reaches a bronchus in communication with the outer world. Soon the organisms are occurring in the sputum of the victim ready to infect another.

Prevention.—The hopeful aspect of the subject is that tuberculosis is a preventable disease. It is questionable whether it will ever be possible to prevent infection. Some workers even argue that this should not be attempted unless we are able to annihilate the tubercle bacilli. However, all agree that every known means should be used to prevent the breaking-down of natural immunity by massive doses of the virulent organism. This implies the prevention of the freshly infected excretion of one individual reaching the body of a second. The sputum of the tuberculous person should be collected and burned. The tuberculous patient going about spitting right and left may be just as dangerous to a community as a maniac running amuck with a machine gun. It is well to remember that the great enemy of germ life is sunshine; consequently, the sun should enter all places where people congregate.

Bovine tubercle bacilli are infectious to man; consequently, they should be prevented from entering food. This can be successfully accomplished by the systematic testing of all animals for tuberculosis and the destruction of the diseased. In the District of Columbia the extent of tuberculosis among cattle has been reduced from 18.87 per cent in 1910 to 0.63 per cent in 1919. Moreover, since the tuberculin test has come into general use there has been a consistent decrease in the number of tuberculous animals which come to the slaughter house, as may be seen from the following:

Year	Percentage	
	Cattle	Swine
1917	0.50	0.19
1918	0.37	0.17
1919	0.33	0.15

Inspection alone cannot be depended upon, but the public must be educated to eat only well-cooked meats even though they have been inspected. All milk should be pasteurized before

being used. The value of this procedure is reflected by the decrease in bovine abdominal tuberculosis in New York. During

FIG. 113.—A visible tuberculosis cow. *(Courtesy of Dr. Frederick.)*

FIG. 114.—Another tuberculosis cow; her appearance would indicate nothing wrong, but the disease was detected by the intradermal test. *(Courtesy of Dr. Frederick.)*

the years previous to the pasteurization of its milk supply 64 per cent of the cases of abdominal and lymph-node tuberculosis were of the bovine type. Today 98 per cent of the city's milk

supply is pasteurized, and only 16 per cent of the cases of lymph-node and abdominal tuberculosis are due to the bovine germs. Malnutrition is the friend of the tubercle bacilli, as it reduces natural immunity. It is well to remember that much mulnutrition is due to poverty, but still more is due to ignorance. It is not sufficient that the child, or the adult for that matter, fill his stomach three times a day, or that he receive the required number of calories; but, as pointed out by McCollum, the diet must contain certain dietary constituents which cannot be obtained from the muscle meats, highly refined grains, and fleshy tubers. The properly balanced diet should contain milk, a leafy vegetable, and a fresh fruit. Milk and leafy plants are especially important in furnishing calcium to the body. Considerable evidence has accumulated within recent years which shows that an abundance of calcium in the diet protects against tuberculosis. Workers who toil in atmosphere laden with calcium-rich dust show a lower incidence to tuberculosis than laborers in other occupations.

It should be constantly borne in mind that debilitating conditions, such as insufficient exercise, overwork, worry, alcohol, and all excesses, lower natural immunity. One should sleep in well-ventilated rooms and avoid dust and other sources of irritants to the lungs. Regular systematic exercise should be taken; well-developed chests are a good insurance against tuberculosis. Colds and pneumonia should receive prompt attention. In short, a simple, regular, well-rounded life should be lived.

Cure of Tuberculosis.—Tuberculosis is one of the most curable diseases known to man, as is readily seen from the fact that from 80 to 90 per cent of the population become infected at one time or another during the course of their lives, and yet only 8 per cent die of tuberculosis. Each year the hopes of many sufferers are caused to burn brighter by the announcement of a sure panacea for tuberculosis, but each year sees the same individuals crushed with disappointment; consequently, today it must be stated that no drug is known which cures tuberculosis. At the present time, hopes are entertained that some gold salt may be found to effect cures, but as yet the conservative workers still counsel the use of the old tried and proven remedies—rest, fresh air, sunshine, proper food, and contentment. These are the five essentials on which humanity today must stake its fight in the prevention of infection, and on these those ill with the disease must hang their hope for recovery. When properly applied they work miracles.

The term "tuberculosis" often strikes terror to the heart of man when applied to him or his loved ones, but the kindest act a physician can do for his patient is to notify him early if tuberculosis is even suspected. Consequently, one of the most potent factors in its cure is the annual medical examination by a competent physician, and if tuberculosis is even suspected one should follow a carefully regulated hygienic regimen in which fresh air, rest, sunshine, proper food, and contentment are the medicines.

CHAPTER XXXVII

DIPHTHÉRIA

The Utopian dream of the bacteriologist will have been attained when he has learned to know the definite microörganism which causes each specific disease; when he has found an exact method for diagnosing the disease, so that he can tell when the patient is no longer a danger to the community and consequently can be released from quarantine; and when he has found a preventive and a curative agent for each specific bacterial disease. All this has been accomplished in the case of diphtheria.

History.—Centuries before the Christian Era diphtheria periodically visited the human race, swiftly took its toll, and left man gazing in utter helplessness. There is good evidence that it existed in Greece long before the days of Hippocrates and it was believed by the Greeks to have come from Egypt. Galen refers to the false membrane that is found in the throat of the patient and which is often dislodged by coughing, and Aretaeus gave a fairly accurate description of the disease as we now know it. However, it was Bretonneau (1775–1862) who laid the firm foundation on which modern work has been done. He showed that diphtheria is an infectious disease and is often spread by the common drinking cup and other table utensils. He was the first to successfully relieve the patient by opening the trachea. Klebs, in 1883, first called attention to a very characteristic microörganism which occurred in the throats of diphtheria patients. The next year Löffler obtained the germ in pure culture and grew it on laboratory media. He was unable to find it in the throats of all diphtheria patients and occasionally found it in the throat of healthy persons; consequently, he concluded that the discovered organism was not the direct cause of the disease. Four years later the mystery was solved when Roux and Yersin triumphantly proved that the Klebs-Löffler bacillus produces a soluble toxin which, when separated from the bacterial body and given to a susceptible animal, reproduces the disease with singular fidelity.

To the distinguished German, Emil Behring, a student of Koch, and to the equally distinguished Frenchman, Emile Roux,

a student of Pasteur, belong the honor of discovering the life-saving antitoxin, which may be used both for the cure of the disease, and the production of a short immunity. In 1913 Schick developed a method for determining when a person has sufficient antitoxin within his body to protect him against the disease in case of exposure. Within recent years the patient, skilled, and scientific work of Park and his associates has developed a method by which a susceptible person may be given a mixture of toxin-antitoxin and thus develop a high degree of lasting immunity. Consequently, all that remains to be done in order to eradicate diphtheria is to educate the masses, so that they will take advantage of the life-saving fruits of science.

Prevalence.—Diphtheria epidemics occur in cycles. The interval between and the severity of epidemics vary widely. This has been noted for centuries, and in 1613 it was so prevalent and so deadly in Spain that in the chronicles of that time this is known as the "diphtheria year." The epidemic years in Boston were 1863–1864, 1875–1876, 1880–1881, 1889–1890, and 1894; in New York 1876–1878, 1880–1882, 1886–1888, and 1893–1894 in; Chicago 1860–1865, 1869–1870, 1876–1879–1881, 1886–1887, and 1890. Various explanations have been offered to account for this phenomenon. Rosenau considers the cycles due to a combination of three factors: (1) man, (2) the bacillus; and (3) the environment. An epidemic may sweep through a city and practically all the susceptible individuals contract the disease. It would, then, be a number of years before there would be another susceptible group of children. There is also the likelihood that the strain of diphtheria germ may be more virulent at one time than at another. Attempts have, also, been made to correlate diphtheria epidemics with the weather, for it is possible that some weather conditions are more instrumental than others in rendering children susceptible to the disease.

The average annual death rate in the United States due to diphtheria before the days of antitoxin was approximately 100 for every 100,000 population. If this ratio had continued to the present time we would be losing annually 100,000 from diphtheria. In 1900, just five years after the discovery of antitoxin, the death rate had dropped to less than one-half of what it was before the days of antitoxin. Since 1900 there has been a gradual and consistent decrease.

The following results indicate clearly the effect of diphtheria antitoxin on the diphtheria death rate.

NUMBER OF CITIES WITH DIPHTHERIA DEATH RATES

Year	40 and over	20 and over	10 and over	Under 10
1890–1894	49	49	51	2
1895–1899	32	48	55	0
1900–1904	18	42	54	1
1905–1909	2	35	54	3
1910–1914	1	33	51	13
1915–1919	0	22	50	18
1920–1923	0	17	52	16

In the first period, 1890–1894, there were 49 cities in the United States with an annual diphtheria death rate of forty or over and only two under ten. By 1915–1925 there was not one city with an annual death rate of over 40, and 16 were below ten.

Today the mortality from the disease depends largely on the locality. It is lowest in the country, for there a person is less likely to contract the disease. It is highest in the small cities where the disease is prevalent and antitoxin is not promptly administered. The average death rate from diphtheria in fourteen large cities as compared with that of the rural districts for the years 1911–1917 is given below:

DEATH RATE PER 100,000 POPULATION

Year	Registered area	Average	
		In rural districts	14 large cities
1911	18.9	15.1	20.5
1912	18.2	15.5	18.7
1913	18.8	14.7	23.4
1914	17.8	13.8	24.0
1915	15.7	12.9	19.8
1916	14.5	11.7	19.2
1917	16.5	12.3	22.7

The death rate from diphtheria in some small cities is nearly as high as it was in the pre-antitoxin days. That is, if it were not for the diphtheria antitoxin the mortality from the disease would be what it used to be in the seventeenth, eighteenth, and nineteenth centuries, as there is no indication of a weakening of the diphtheria virus. However, the cities have shown a consistent

decrease even down to 1926, as may be seen from the following table in which are given the total diphtheria death rates for 70 cities from 1923–1926:

Year	Population	Diphtheria deaths	Diphtheria death rate per 100,000
1923	29,243,128	3,838	13.12
1924	28,810,993	3,285	11.05
1925	31,049,595	3,024	9.74
1926	31,486,891	2,988	9.48

The Cause.—The diphtheria bacillus, the organism, which causes diphtheria, varies considerably in its general characteristics. These depend largely on whether the organism has been grown on laboratory media, or whether it has come directly from the throat. When grown on Löffler's blood serum it possesses characteristics definite enough, so that it can be morphologically identified, a condition rarely met in bacteriology. The size, morphology, and staining properties vary greatly with different races of the organism. They are all slender, straight, or slightly curved rods varying in diameter from 0.3 to 0.8 micron, and in length from 1.5 to 6 microns. They may occur with club-like enlargements at one or both ends, or equatorial enlargements with ends more or less pointed. Occasionally, only one end is thickened, giving rise to a long somewhat wedge-shaped rod. The club-shaped organisms are rarely seen in films made direct from the false membrane, but they often occur in cultures grown on laboratory media. Although some strains of the diphtheria bacilli stain uniformly, it is more common to find an alternate arrangement of dark and light bands, giving them a beaded appearance, and in extreme cases making the organism resemble the streptococci. The irregularity of staining is well brought out by Löffler's alkaline methylene blue. In many cultures, properly stained, there can be seen situated at the equator, or near the ends, round, or oval bodies. These are

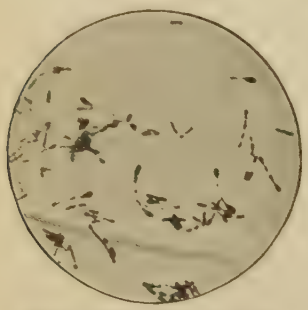


FIG. 115.—Corynebacterium diphtheriæ. × 1100. (*Park.*)

stained more intensely than the rest of the body, and at one time were believed to indicate a high degree of virulence on the part of the organism containing them; hence, special care was used in their determination. However, today it is generally believed that they are of the same significance as the metachromatic granules in other bacteria.

The diphtheria bacteria have been divided into the following classes, according to the manner in which they take certain stains: (1) The granular type, which stains in irregular patches or blocks with the intervening part only slightly stained or unstained. This type predominates in clinical diphtheria. (2) The barred

Fig. 116.—Wesbrook's types of corynebacterium diphtheriæ. (*From Jordan, General Bacteriology.*)

type which when stained has a striated appearance. This type is found both in diphtheria and healthy throats. It is sometimes stated that this is the sole type found in some cases of diphtheria, but Jordan considers this to be untrue. (3) The solid type which stains uniformly. Some authorities class these as pseudo-diphtheria bacilli and do not regard them as capable of causing disease. However, there is some evidence that one type may change into the other. Consequently, Park and Williams consider the only practical method of differentiating virulent from non-virulent diphtheria germs to be by animal inoculation. They have found a few strains of virulent diphtheria bacilli which fail to show a marked characteristic stain and quite a

number of pseudo-diphtheria organisms which show dark bodies. The important factor, however, is that all virulent strains are controlled by the same antitoxin.

The diphtheria bacillus grows on ordinary laboratory media, provided a suitable temperature is maintained (17° to 43° C., with an optimum of 37° C.). On Löffler's blood serum it produces characteristic colonies and organisms. On this media the diphtheria bacilli outgrow the common parasitic organisms found in the mouth; consequently, this is used in the diagnosing of the disease. Diphtheria bacteria possess no capsule, are non-motile, form no spores, but are more resistant than most non-spore-bearing bacteria. They may remain alive in dried false membranes at room temperature for several months, or for one hour at 98° C. They are killed in ten minutes at 60° C., five minutes at 70° C., and almost instantly in boiling water. They are quite resistant to disinfectants while in the false membrane, but in the free condition they are readily killed by the ordinary disinfectants. Hydrogen peroxide is said to be especially effective against them.

Toxin Production.—The diphtheria bacillus is one of the few bacteria which produces a highly potent exotoxin. No two strains have the same power of producing toxin and some lose their ability quickly when grown on laboratory media. The one isolated by Park in 1895 is now generally used for toxin production, and it is just as powerful today as when first isolated. Toxin is readily produced by growing the organism in beef tea. The presence of carbohydrates inhibits its production; hence, toxin is not produced in milk. Although pure toxin has never been obtained, yet, some very poisonous solutions have been prepared. The toxin is believed to be proteinaceous in nature. It s destroyed by a temperature of 58° to 60° C. and is rendered inactive by the digestive juices; but when injected into experimental animals it causes death, paralysis, or the production of anti-bodies, with a resulting immunity, depending upon the quantity given. The production of the toxin and antitoxin has already been considered on page 380.

Modes of Transmission.—The diphtheria bacillus leaves the body in the secretions of the nose and throat. These secretions are, consequently, the infecting agents. The organism may be transmitted directly from one individual to another by droplets, fingers, toys, pencils, spoons, cups, handkerchiefs, and the many other objects which are placed in the mouth of the infected child

and later find their way into the mouth of a healthy person. The organisms may come from the throat of active cases, recent convalescents, mild and missed cases, and carriers, and is spread by the rather quick exchange of microörganisms from the mouth of one to that of another.

Carriers.—Löffler, in his first publication on diphtheria, referred to the fact that in the course of the examination of the throats of twenty children he found in the throat of one, bacilli which were indistinguishable from the true diphtheria germ; nevertheless, this child had never suffered with the disease. This was used as evidence that Löffler had not isolated the cause of the disease. It was learned during the nineties of the last century that individuals who have recovered from the disease, or even others who have never had diphtheria may carry within their throats the diphtheria germ. In 1893–94 there were studied in the Health Department of New York City 752 cases with the following results:

In 43.2 per cent of the cases the diphtheria organism disappeared in 3 days.
In 26.7 per cent of the cases the diphtheria organism disappeared in 5 to 7 days.
In 11.1 per cent of the cases the diphtheria organism disappeared in 12 days.
In 9.1 per cent of the cases the diphtheria organism disappeared in 15 days.
In 7.5 per cent of the cases the diphtheria organism disappeared in 3 weeks.
In 1.4 per cent of the cases the diphtheria organism disappeared in 4 weeks.
In 0.6 per cent of the cases the diphtheria organism disappeared in 5 weeks.

From this and similar work it is often stated that the bacilli begin gradually to disappear from the body of convalescents, and by the end of a month 85 per cent of the convalescents are free of the bacilli. By the end of the second month 98 per cent are free, and the remaining 2 per cent pass into the chronic-carrier state. Foreign bodies, or deformities, in the nose and throat predispose to the chronic-carrier state. It has been further found that from 10 to 20 per cent of the individuals who come in contact with diphtheria patients harbor the germ in their throats. In some 80 per cent of such cases they are harboring the virulent organism, and although they do not contract the disease themselves, they are a danger to the community. Extensive studies indicate that about one in 1,000 of the general population is a carrier of virulent diphtheria germs. However, among children, about 2 per cent are carriers, and about 10 per cent of these harbor diphtheria germs capable of causing the

disease. Often considerable difficulty is experienced in trying to free chronic carriers of the germs. This is really one of the big problems which confronts the health worker, for were it not for the carriers the disease would be much less of a problem. It is these individuals that normally keep the disease alive and greatly facilitate the spread during epidemics.

The Schick Reaction.—For some time it was not understood how the healthy individual could carry the diphtheria germ and not come down with the disease. In 1913 Schick developed a method by which could be determined those individuals who had within their blood sufficient antitoxin to protect them against diphtheria. A minute quantity of the diphtheria toxin is given intracutaneously, and a local reaction follows if there is less than one-thirtieth of a unit of antitoxin in a cubic centimeter of the individual's blood. This quantity is sufficient, under ordinary conditions, to protect against the disease. The application of this test to carriers showed that they carry the antitoxin. The test has been applied to over 20,000 individuals with the following results:

Age	Average susceptible (per cent)
At birth	10
Under 4 months	15
4 to 6 months	30
6 to 9 months	60
9 months to 1 year	75
1 to 2 years	75
2 to 3 years	65
3 to 5 years	40
5 to 10 years	30
10 to 20 years	25
Over 20	20

The susceptibility to diphtheria is low at birth, but it gradually increases with the age of the individual. It becomes highest between the ages of one and two years, after which it decreases. Only about 20 per cent of the adults are susceptible to diphtheria. The results indicate that immunity goes in families, and the children of some nationalities are more immune than other children. In the city of Auburn, Sears found the positive reactions among the Polish children to be 26 per cent, among the Italian children 36 per cent, and among the English-speaking races 82 per cent; the general average for all races being 60.5

FIG. 117.—Shows four typical positive Schick reactions of varying degrees of intensity forty-eight hours after test. (*Zingher*, "*American Journal of Diseases of Children*," *April*, 1916.)

per cent. The great practical significance of the test rests on the indication that certain individuals are not immune and should receive preventive treatment.

Prevention.—During the nineteenth century, diphtheria showed a definite, although irregular increase. This was probably due to the greater crowding of individuals into highly populated centers. It showed little reduction when better sanitation was instigated. The knowledge that the disease is communicable, the identification, and the isolation of the specific germ causing the disease did not help much. Soon after the discovery of the antitoxin, in 1895, there was a decided drop in the mortality, but it had little effect on the number of cases. In New York special pains were taken to prevent convalescent carriers from being released from quarantine and to search out many healthy carriers. Still the results were disappointing; consequently, it became evident, laudable as were the campaigns against the placing of infected objects in the mouth, the careful quarantining of diphtheria cases, and the systematic use of antitoxin, that diphtheria could be controlled only when it was once learned how to effect a permanent immunity to the disease.

During the first decade of the twentieth century a number of workers had used mixtures of toxin-antitoxin to immunize lower animals, and several had suggested its use for the immunizing of children. Yet, it was not tried out until 1913, first by Behring, and later by Park. The latter found that by taking definite quantities of an appropriate mixture of toxin-antitoxin a high degree of lasting immunity could be produced in children. The perfecting of the Schick test in 1913 gave an efficient method for determining those susceptible to diphtheria, and a method of determining when the toxin-antitoxin-treated child was immune.

The results of this work indicate that toxin-antitoxin treatment gives rise, in one to three months, to an immunity which lasts for at least ten years and probably life. Inasmuch as the immunity does not develop for some time after the treatment, it is valueless in those cases exposed to diphtheria. In such cases the regular antitoxin is used; consequently, it is held today by authorities that if all children were systematically tested by the Schick method and the susceptible ones given the toxin-antitoxin treatment, diphtheria could be completely eradicated. During the four years previous to the use of toxin-antitoxin on the school children of Auburn, a city in New York with a population

of 36,000, the average annual death rate from diphtheria was 14, and the number of cases 104. Three years of systematic work with toxin-antitoxin reduced this to three cases with no deaths. The results obtained by Park, in his anti-diphtheria campaign, which is being conducted in New York, are of the same nature, as is shown by the following results:

DIPHTHERIA CASES AND DEATHS IN NEW YORK CITY
(1919–1923)

Year	Cases	Deaths
1919	14,014	1,239
1920	14,166	1,045
1921	15,110	891
1922	10,427	874
1923	8,050	547

The cases have been reduced nearly one-half and the deaths over one-half in five years. These favorable results are attributed by Park to the combined efforts of the health department, medical inspectors, who were instrumental in giving immunizing antitoxins, and private physicians, as well as to the general education of the people of the city and state of New York. It was primarily these two factors that have brought about this striking reduction in morbidity and mortality. All are agreed that we have the means of ridding communities of diphtheria; consequently, today the diphtheria problem is similar to the smallpox problem, and depends upon the education of the masses to the safety and efficiency of diphtheria immunization. The reasons it is better to prevent diphtheria with toxin-antitoxin treatment than to depend merely upon proper treatment are thus summarized by Nicoll:

"(1) Antitoxin may be administered too late and in insufficient quantity to save life. Evidence of diphtheria may be so slight that a physician is not called early enough.

"(2) One form of diphtheria (laryngeal) attacks the larynx, or windpipe, and may cause croup but no sore throat. The child may choke to death before medical attendance can be had.

"(3) Another form, nasal diphtheria, attacks the lining of the nose, and may be regarded as a common cold until it is too late.

"(4) A person who has diphtheria must undergo a period of illness and may suffer from bad after-effects, especially heart disease.

"(5) Other members of the family must usually suffer inconvenience from quarantine regulations."

Antitoxin.—The sovereign remedy for the cure of diphtheria is the antitoxin, for before the days of antitoxin there were diphtheria epidemics in which the mortality was 35 per cent or over. Now, when antitoxin is given during the first signs of the disease, the percentage of recoveries is 100 per cent. The antitoxin should be administered on the very first symptoms, for every hour counts. Hilbert collected data on 2428 cases and found that the percentage of deaths varied with the day on which the antitoxin was used the results are as follows:

Time antitoxin was administered	Percentage of deaths
First day	2.2
Second day	7.6
Third day	17.1
Fourth day	23.8
Fifth day	33.9
Sixth day	34.1
After sixth day	38.2

It has been estimated that through the use of antitoxin in the United States annually 20,000 lives are saved, and that if properly and promptly administered the annual saving in Germany alone would be 45,000 lives.

In addition to this, there are the still larger numbers who are saved from contracting the disease when given the antitoxin on exposure, as antitoxin is not only a curative but a short preventive; consequently, it is used in two sets of cases. (1) It is used in connection with those suffering with the disease; and (2) with those exposed to the disease and who have not been immunized by the toxin-antitoxin mixture. There is practically no danger from the use of the antitoxin, and when properly used means a speedy recovery from diphtheria. Occasionally it happens that individuals, who suffer from the disease and are given the antitoxin, die, or are left paralyzed, or otherwise seriously injured. However, in no case are these effects to be considered

due to the use of the antitoxin. They simply indicate that the antitoxin was administered too late, and that the disease had done so much damage before the antitoxin was given that the individual would have died or never have completely recovered anyway. When insufficient quantities of antitoxin are given the lives of the patients may occasionally be saved, but because the amounts were not sufficient to neutralize all the toxin, the individuals are left permanently injured after recovery.

CHAPTER XXXVIII

TETANUS

Tetanus, or lockjaw, as it is commonly called, is a comparatively rare disease; nevertheless, due to its peculiar spasmodic symptoms and its high mortality, it early attracted special attention. The discovery of the causative organism intensified this interest. It is caused by an anaërobic highly resistant spore-producing saprophyte. It enters the body through a wound and elaborates, at the point of entry, a highly potent toxin which damages primarily nervous tissue.

History.—It is evident from the writings of Hippocrates and his contemporaries that tetanus as well as some other diseases that have been mentioned were prevalent and recognized before the beginning of the Christian Era. The Arabian physicians during the ninth and tenth centuries accurately described the disease, but they, like their predecessors, attributed it to sudden changes in the weather, the wetting and chilling of the body. The advancements in the field of medicine are mirrored by the interpretations placed upon this disease. It ran the gauntlet of the prevailing theories of the ages—evil spirits, improper humors, miasma, noxious effluvia, taking cold, rheumatism, and finally the germ theory. Each, at one period or another, was pressed into service to explain tetanus. About the middle of the nineteenth century the belief that it was an infectious disease commenced to crystallize. From 1868 to 1870 numerous attempts were made to infect experimental animals with tetanus by blood from men suffering with the disease. As can be readily understood today, the results were all negative. In 1884 Carle and Rattone for the first time produced the disease experimentally. They demonstrated that the disease can be transmitted from the wound of one animal to that of another. The following year Nicolaier, led on by the observation that gardeners are prone to contract the disease after slight injuries to the feet and legs, produced it experimentally in a number of small animals by inoculating them with garden soil. He was able to demonstrate that in the pus of such wounds were drumstick-shaped bacilli. Four years later Kitasato recognized

this enlargement of the bacillus to be the result of a spore; consequently he heated it, killed all vegetative organisms, and thus obtained the germ in pure culture. This he inoculated into experimental animals and found that they contracted tetanus. The following year Faber discovered that a filtrate of the media in which the bacilli had grown, but from which their bodies had been completely removed, caused the disease. About this same time Behring and Kitasato prepared an antitoxin and demonstrated its value.

Prevalence.—Inasmuch as the wounded soldier occasionally contracts tetanus, it is often stressed in relation to war. Accurate statistics are not available, yet an idea of its prevalence may be gained from the following table:

	Number wounded	Cases of tetanus	
		No.	Percentage
Crimean War	12,094 (English)	19	0.15
American Civil War	217,000	505	0.20
Russo-Turkish War	51,700 (Russian)	66	0.12
World War	27,677 (German)	174	0.62
World War	380,000 (English)	292	0.08

Among the wounded sent to England during the World War there were about 1,450 cases of tetanus, and it has been estimated that were it not for the early systematic use of tetanus antitoxin there would have been 14,000 cases.

It is, however, not in war but in peace that this germ has taken its greatest toll. Before the days of aseptic surgery, and in some localities even at the present time, the organism entered the newborn infant through the umbilical wound. Among the negro babies in certain of the West Indian Islands tetanus has had a mortality as high as 50 per cent. In 1856 four per cent of all deaths in Charleston and 3.7 per cent of all deaths in New Orleans were caused by this disease. It has often taken a terrible toll among the negro children. In Venezuela it is one of the chief causes of infant mortality. One author states that at one time one-fourth of all the children born in Rio de Janeiro died from tetanus.

Until the gospel of a sane and safe Fourth of July had been vigorously preached, there was, as is shown in the following table, the annual July crop of deaths from tetanus in the United States:

Year	Number of cases of tetanus
1903	406
1904	91
1905	87
1906	75
1907	73
1908	76
1911	10
1916	0

As a result of the vigorous campaign waged by the American Medical Association for sane Fourth of July celebrations, and the early use of tetanus antitoxin this loss has been checked.

Occurrence.—The tetanus bacillus is widely distributed in nature. Its normal habitat appears to be the alimentary tract of herbivora; hence, it occurs in the droppings of horses, cows, and many other animals. It frequently occurs in the feces of man, and it is usually stated that it is more prevalent where individuals are around horses. The following table illustrates the extent to which it has been recovered from human feces:

Investigator	Percentage	
Pizzini	5	
Tullach	33	(Soldiers returning from France)
Tullach	16	(English civilians)
Ten Broeck and Bauer	34.7	(Chinese)
Ten Broeck and Bauer	26.5	(Chinese)
Buzello and Rahmel	40	(Germans)
Kalm	12.5	(New York)
Bauer and Meyer	24.6	(California)
Bauer and Meyer	21	(Country at large)

The number of determinations in some cases was low; consequently, the error is large, if one tries to apply this to mankind as a whole, but it is suggestive and seems to indicate that about

one-fifth of the population are tetanus carriers. As the results indicate, there is a wide variation depending on the locality. Individuals in some districts would ingest the tetanus spores oftener than would individuals in other districts. The results establish the following points: (1) Human carriers are not limited to persons associated with horses and cows; and (2) human feces may play a prominent part in infecting soil.

The organism is widely distributed in soil, especially garden soils. It has been obtained from hay, clothing, the skin, old masonry, fire crackers, gun wads, gelatin, and catgut. LeDantic reports having found it on the poisoned arrows of the natives of the New Hebrides, who obtained it by smearing their arrowheads with the material found in the burrows of crabs. In a few cases it has accidentally found its way into vaccines and serums with fatal results. However, the precautions taken at the present time in the manufacturing of these products will most probably prevent its recurrence.

Recently a number of fatal cases of tetanus have resulted in the United States from the use of bunion pads as vaccination dressings. Laboratory tests showed 25 per cent of the bunion pads examined to carry tetanus bacilli. Deaths from this source are entirely unnecessary, for even superficial wounds should be dressed and covered with sterile dressing. Furthermore, the use of a shield of any form in vaccination is to be discouraged, since it tends to prevent evaporation, retains the moisture, heat, and exudates, and consequently, softens the vesicle and renders conditions better for bacterial invasion.

Tetanus bacilli are more prevalent in warm than in cold districts, although Iceland at one time suffered severely from this disease. The soils of the Atlantic States, as well as the soils of Flanders and France contain great numbers. Apparently, wherever the feces of man and the lower animals reach the soil large numbers of tetanus bacilli may be expected.

Properties.—The tetanus bacilli are long, comparatively slender, rod-like organisms with rounded ends. They measure from 0.3 to 0.8 micron in diameter and from 2 to 6 microns in length; usually occur singularly, or in pairs, although long chains sometimes occur in cultural media; are slightly motile, with flagella surrounding the body; and no capsule but produce spores. The spores formed are produced in the end of the mother cell and have a diameter greater than the bacilli; consequently, while they are in the body of the mother cell they give rise to a drum-

stick-shaped organism which at times resembles minute pins. They are highly resistant and survive in flowing steam for 40 minutes, and occasionally for 60 minutes. They may survive a dry temperature of 125° C. for 20 minutes and remain alive in dry soil for many years. Henrijean found them to germinate after eleven years of drying. A 5-per cent solution of carbolic acid destroys them in about 15 hours, but if 0.5 per cent hydrochloric acid is also added, they are killed in two hours. Mercuric chloride (1 to 1,000) kills them in about three hours, and a 1-per cent solution of silver nitrate in one minute. Iodoform is said to be very effective in preventing germination.

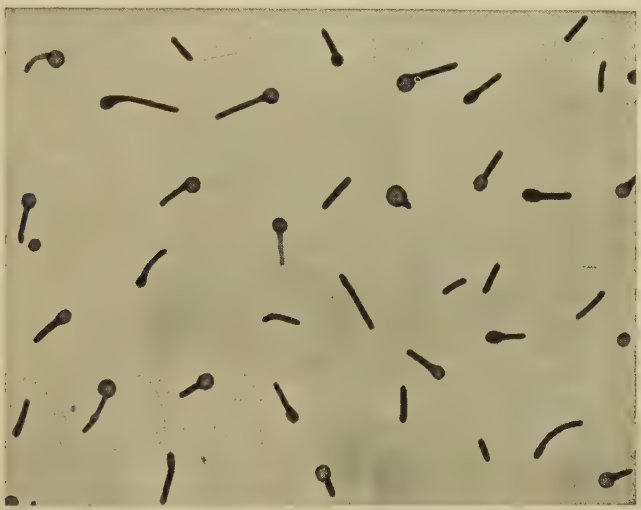


FIG. 118.—*Cl. tetani* showing spores. Eleven-day culture in plain agar stab, stained with gentian-violet. (*From Ford, A Text-book of Bacteriology.*)

The tetanus bacillus is an anaërobe with an optimum temperature of 37.5° C., under which conditions it grows well in ordinary laboratory media. If planted with certain aërobes it grows well even though oxygen has access to the surface of the media. It stains readily with the common analine dyes.

Pathogenicity.—Mice, guinea pigs, rabbits, horses, cattle, goats, and men are highly susceptible to tetanus. Rats and birds are less so, and fowls scarcely at all. The cold-blooded animals are immune, although it is stated that they may be infected by raising their temperature. It is generally stated that

if one gram of horse requires one part of toxin to kill, then one gram of guinea pig requires 6 parts; one part of mouse, 12; of goat, 24; of dog, 500; of rabbit, 1,500; of cat, 6,000; and of hen, 360,000. It is surprising to find that the tetanus bacilli multiply in the alimentary tract of the horse, yet a quantity of toxin sufficient to kill a hen would suffice to kill 500 horses.

The tetanus bacilli do not circulate in the blood but elaborate their toxin at the point of entry. This, in turn does the damage to the various tissues of the body, especially the nerves. Pure cultures of the organism, free from toxin, when injected into experimental animals are usually rather quickly destroyed. But if the tetanus bacilli, together with some of their toxin, or even other irritants are used, tetanus is likely to follow. The likelihood of tetanus varies with the wound. Deep or infected wounds, and those in which the surrounding tissue is destroyed, or even greatly damaged are more likely to develop tetanus if the organism is present, than clean-cut, non-suppurating wounds. However, the severity of the wound does not influence the likelihood of tetanus. It may result from pin pricks, nail punctures, insect bites, vaccination, and many other mild injuries. It is evident that the likelihood of tetanus is always present in dirty wounds. Covering even superficial wounds with adhesive tape, or collodion produces anaërobic conditions that may become favorable for the development of the tetanus bacilli.

Toxin.—The tetanus bacilli in appropriate media elaborate a very potent toxin. The growth of the organism in beef tea free from glucose has resulted in a solution so highly toxic that 0.0000005 cc. will cause the death of a mouse, and purified solutions have been obtained one hundred times as toxic. It is estimated that the toxin produced by the tetanus germ is twenty times as poisonous as dried cobra venom. The potency of the toxin formed varies with different types of the organism, but fortunately all are neutralized by the same antitoxin. Tetanus toxins are composed of several poisons. The best known are tetanolysin which destroys cells, and tetanospasmin which causes the spasms. These toxins are destroyed by heat, sunlight, and oxidation but may be precipitated and kept in a vacuum in the dark for long periods. If taken by the mouth the toxin is harmless, but if injected into susceptible animals it causes severe nerve injury. The time elapsing between the administering of the toxin and the appearance of the symptoms in mice has been found to vary with the dose.

1 fatal dose	Symptoms 4 to 7 days
2 fatal doses	Symptoms 2 to 3 days
10 fatal doses	Symptoms 36 to 48 hours
300 fatal doses	Symptoms 20 hours
3,000 fatal doses	Symptoms 12 hours
30,000 fatal doses	Symptoms 10 hours
90,000 fatal doses	Symptoms 9 hours

It appears that time is required for the toxin to reach and injure the tissues and this cannot be obliterated by even massive doses. In this respect bacterial toxins differ from many other poisons.

The incubation period in man, counting from the time the wound is received until symptoms appear, has been found to vary from two days to one year. However, it is usually from four to seven days.

Antitoxin.—The tetanus antitoxin is more effective in preventing than in curing tetanus. Although, according to some authorities, its systematic use, even after symptoms have developed, has reduced the mortality from about 80 to 40 per cent, its failure to always effect a cure is probably due to the fact that the toxin has a greater affinity for the nervous tissue than it has for the antitoxin. It was shown that if a mixture of toxin and antitoxin was made before injecting into an animal, twelve fatal doses were neutralized by 1 cc. of a 1-to-2000 dilution of antitoxin. If the antitoxin was administered four minutes after the toxin, 1 cc. of a 1-to-600 dilution was required; if eight minutes after, 1 cc. of a 1-to-200 dilution; if fifteen minutes after, 1 cc. of a 1-to-100. Hence, if tetanus is to be prevented it is desirable that the antitoxin be administered at the earliest date possible after the wound is received.

Prevention.—The prevention of tetanus rests upon three factors: (1) The prevention of wounds insofar as possible, for it should always be borne in mind that even the breaking of the skin is a welcome to microörganisms. Unnecessary wounds from nails and other objects occur entirely too often. (2) All dirty wounds should be carefully cleaned and then properly dressed. During the war the best method of cleansing wounds was found to be with the surgeon's knife. All dirt, bits of clothing, foreign material, and destroyed tissue should be removed. The wound should be dressed in such a manner that air has access to it. (3) When dirty, highly infected wounds of such a nature that tetanus may result are received, antitoxin should be administered by a competent physician.

CHAPTER XXXIX

TYPHOID

An examination of mortality tables convinces one that some parts of the human body are more vulnerable to the attacks of microörganisms than others. Approximately 30 per cent of the annual deaths in the United States are caused by the breaking-down of the respiratory system, and 23 per cent to a breaking-down of the gastro-intestinal system. Although this is not completely due to microbial activities it is evident that these two systems are much more exposed to the vicissitudes of the environment than the other systems are. Most bacteria enter the body by the respiratory, or digestive routes and often locate in one system or the other and cause the breaking-down of the whole body. Tuberculosis and pneumonia have already been considered as types of the respiratory diseases. Typhoid will now be taken to represent the gastro-intestinal group of diseases. This group is composed of typhoid, para-typhoids, cholera, and the dysenteries, all of which are quite closely related. Practically all of these diseases are peculiar to man and most prevalent during hot weather. The causative organisms enter the body through the mouth and leave in the alvine discharges. They breed primarily in the human body; consequently, man is the fountain head. Fingers, flies, and food play a prominent rôle in their spread. In the sense that these diseases have yielded to good sanitation to a greater degree than most other communicable diseases, they may all be classed as filth diseases.

History.—The disease now recognized as typhoid or enteric fever was for centuries confused with other lasting fevers. Even today it may be mistaken for septic fever, typhus fever, malaria, tuberculosis, and other lasting fevers unless bacteriological means are used in its diagnosis. Hippocrates, in writing on epidemics, describes certain diseases which he finds are generally accompanied by delirium, disturbances of the bowels, great emaciations, and a fever which sometimes lasts for forty days. He also notes that these diseases are more prevalent at some seasons than at others. The first attempt to distinguish typhoid

fever from typhus fever was made by Thomas Willis in 1659, who found that typhus fever was more contagious than typhoid, caused more eruptions on the body of the patient, and was accompanied with fewer complications than typhoid. From this date on, there were occasionally individuals who believed that typhus and typhoid fevers were two distinct diseases; but even as late as the nineteenth century all continued fevers accompanied with delirium were grouped under the general head of typhus fever, and it was not until the nineteenth century with the work of the French clinicians that the fact that there are two distinct diseases was established. The term typhus was retained for the disease in which there were no intestinal lesions. The term typhoid, which means resembling typhus, was applied to the disease accompanied with intestinal lesions. Pettenkofer had taught that the filth in soil undergoes a ripening process in which the virus of typhoid is elaborated, and the actual spread of the disease he considered due to the fluctuation of the ground water. In 1856 William Budd commenced the publication of a series of articles which laid the foundation for our present knowledge. In these he contended that typhoid is contagious, that the causative organism is found in the dejecta of the patient, and that carriers play a part in its spread. The Typhoid Commission, which functioned during the Spanish-American War, definitely proved that the disease is contagious and may be carried by flies, and that it was typhoid, and not the so-called typhoid malaria, that was "playing hob" with our troops.

The year 1880 marks the beginning of our bacteriological knowledge of typhoid. Eberth, Klebs, and Koch each made independent contributions at this time concerning the causative organism. Four years later Gaffky grew the organism in pure culture but failed to produce the disease by feeding cultures to experimental animals. However, since then enough evidence, some gained from accidental laboratory infections, has accumulated to fully demonstrate that the *Bact. typhosum* is the specific cause of typhoid fever. Other work has shown that the closely related microörganisms, paratyphoid α and paratyphoid β, give clinically similar, but bacteriologically, distinct diseases. Several bacteriological methods for diagnosing the various fevers have also been developed, as well as a highly successful preventive vaccine.

Prevalence.—In the past typhoid has been one of the great causes of death and disability, and in rural districts it is a great

factor even at the present time. The extent of this disease in the United States in 1900 has been estimated at 353,790 with a death rate of 35,379. By 1914 the number of cases had diminished to 198,000 with a death rate of 19,800. In 1925 there were almost 50,000 cases and the death rate was probably about 5000. This is a very favorable showing for the past quarter of a century. The mortality from typhoid fever commenced to drop in many civilized countries in 1880 and has shown a gradual and persistent decrease ever since. The decrease has been more rapid in foreign countries than it has in America, as may be seen from the following figures for 1910:

	Population	Average mortality per 100,000
33 largest European cities	31,500,000	6.5
57 largest American cities	21,000,000	19.59

In this disease the United States, as compared with foreign countries, has been extremely backward in applying recognized methods of sanitary prevention. At the present time the mor-

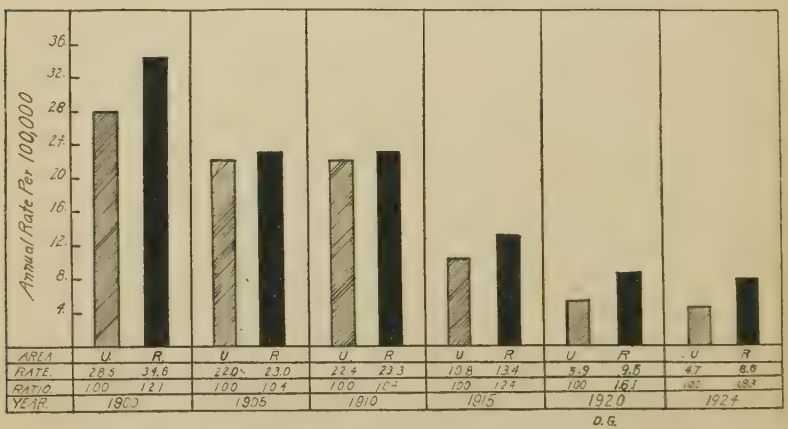


FIG. 119.—Comparative incidences of typhoid in city and rural district.

tality of this disease in the United States in general is not large (6.7 per 100,000), but when this is compared with the average typhoid death rate of the cities (3.07), it is evident that much remains to be done in the rural districts. According to

Rosenau: "In recent years there has been scarcely enough typhoid fever in our large metropolitan centers to furnish material to teach medical students." Typhoid fever within recent years has passed from a disease of the city to a disease of the country and in some places is referred to as a vacation disease, because of the fact that some contract it during their vacations in the country and carry it back to the city.

Appalling as have been the losses from typhoid in civil communities, they have been still greater in the armies. This is shown by the following mortality statistics from Munich—city and garrison.

	Munich city	Munich garrison
1855–1869	204	840
1876–1881	57	190

It is estimated that during our Civil War there were 75,361 cases of typhoid in the army with 27,056 deaths. Typhoid fever accounted for 65 per cent of the total deaths in the Franco-Prussian War. The typhoid losses in the Spanish-American War far exceeded those from the enemies' bullets. Similar conditions existed in the British South African War. In the recent World War, as a result of vaccination, typhoid fever played an insignificant rôle.

The economic loss due to typhoid fever is great. It is a disease which usually attacks robust individuals who are in the prime of life. It is estimated that fully 50 per cent of its victims are between the ages of 15 and 25 years. It is a long-drawn-out disease which not only keeps its victim from his work for long periods, but necessitates the services of others to care for him. Whipple has estimated that each typhoid death is a loss of $6000 to the community. At this rate, the cost in 1900 was over 200 million dollars and in 1925 between 30 and 40 million!

Causative Organism.—Typhoid is often referred to in the plural as there are three similar organisms causing similar diseases: *Bact. typhosum, Bact. paratyphosum* α and *Bact. paratyphosum* β. Morphologically and physiologically the organisms are similar and belong to the colon-typhoid group. Often too, the diseases produced by these organisms are so similar that they can be differentiated only by bacteriological methods.

Singularly too, an attack of one does not protect against an attack of another.

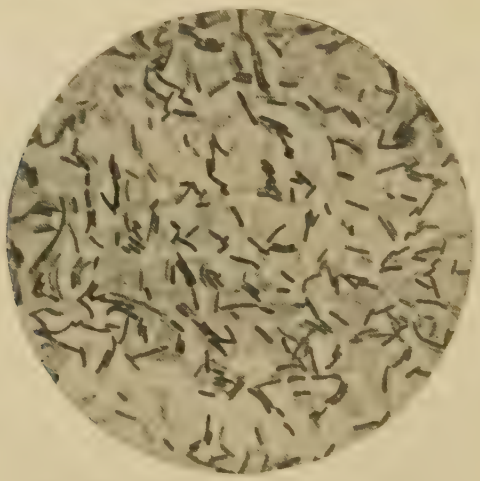


FIG. 120.—Bacterium typhosum. (*Hicks.*)

Paratyphoid seldom occurs as an epidemic, and usually has a shorter incubation period and a lower mortality than is the case

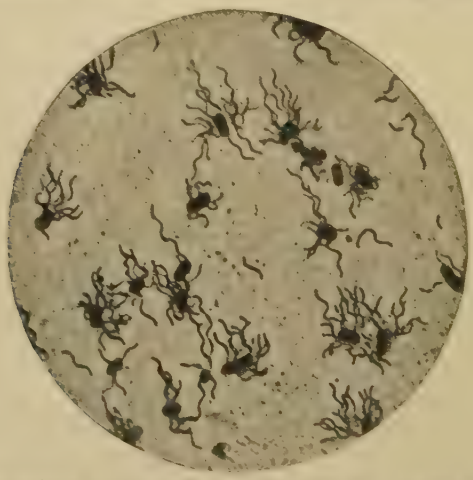


FIG. 121.—Bacterium typhosum showing flagella. (*Fränkel and Pfeiffer.*)

with typhoid. The organisms of these diseases differ in their ability to ferment certain sugars.

The *typhoid bacilli* may be obtained from the rose spots, circulating blood, feces, and urine of the patient during the time he is ill with the disease and from certain of his organs after he is dead. They stain readily with the ordinary analine dyes, and morphologically are short plump rods, varying in length from 1 to 3 microns and in diameter from 0.5 to 0.8 micron. Usually when grown in a liquid they are actively motile and somewhat longer than when they are grown in solid media. These characteristics may be lost temporarily in old cultures or when the media is too alkaline. Under appropriate conditions capsules may be demonstrated.

The organisms grow well on ordinary laboratory media but less readily than *Bact. coli*. Their optimum temperature is that of the human body, but they will grow through a range of from 8° to 44° C. A temperature of 60° C. for ten minutes, or 100° C. for three minutes kills the bacilli. They are highly resistant when in feces. They withstand cold well, survive in ice, and especially in ice cream, for long periods, and they may survive in water from 15 to 21 days, although they usually disappear sooner. They multiply rapidly in milk but not in water, even though it may be heavily laden with organic matter.

Occurrence.—The great reservoir of the typhoid bacillus is man himself, and even when found outside the human body, they can be more or less directly traced to the discharges of a typhoid patient or convalescent. From man it may find its way into soil, water, milk, food, or onto anything handled by man. It multiplies in milk and certain foods, but its multiplication outside the human body is very limited. It finds its way into natural water with sewage, but the times it has been isolated from water are few. It may survive for weeks, but does not multiply in the fecal matter of privy vaults and polluted soil.

Pathogenicity.—The typhoid bacilli naturally attack only man. They enter through the mouth, cause ulcers in the intestines, and pass from the intestines into the blood stream. The period of incubation varies from 10 to 21 days, during which time no symptoms are present. The disease often begins with a headache, listlessness, pain in the legs and back, followed by the characteristic fever. The fever is slight at first, but gradually increases from day to day until it reaches a maximum of 103° to 104° F., and sometimes higher. This is followed by a similar

step-like decline. In the majority of cases rose-red spots appear on the abdomen, and the patient goes into a delirium. It is as if a cloud were cast over the mind. Vaughan very vividly described the effect as he saw it during the Spanish-American War: "Sometimes their eyes turn toward me or toward a comrade. In some, the eyes are full of meaning, bespeaking a brain in action; in others they are dull and staring, indicating benumbed or comatose intellectuality. Some faces show the hectic flush of fever; some the pallor of approaching death; some of the figures are full and rounded, showing but slight departure from health; others are wasted and skeleton-like; some are motionless; others are picking at the bedding, or plucking imaginary objects from the air. All are prostrate on cots, but some are attempting to rise and unreasonably impatient of restraint. The pictures are not all silent ones; some are gentle in speech; others are violent and denunciatory; some are muttering in low delirium; others are shouting in wild mania."

Typical typhoid has been produced in the chimpanzee by feeding milk, or broth cultures of the germ. A form of the disease has been produced by feeding carrots smeared with typhoid bacilli to fasted rabbits. Most animals are unharmed when fed the typhoid germ, but many succumb when the organisms are injected into their tissues, or blood streams. However, the resulting disease cannot be considered as typical typhoid. Fear is sometimes entertained that cows drinking typhoid infected water, may thus infect the milk, but such fears are groundless. The only way in which milk can be infected is from the infected surface of the animal's body. Milk is usually infected by a human carrier.

Routes of Infection.—Typhoid bacilli, under ordinary circumstances, commence to diminish immediately on leaving the human body. This phenomena aids greatly in limiting typhoid, for, when the germs live under unfavorable conditions, each successive link in the chain which connects the first typhoid case with the second generally tends to become weaker and to diminish the danger of infection. It is therefore, not surprising to find that most cases come from the rather rapid transfer of the excreta of the body of one person to that of another. It is impossible to measure exactly the magnitude of each method in typhoid distribution, but the following results are instructive, as they give the percentages of typhoid attributed by various authorities to different causes:

	Contact	Water	Milk
Chapin	10–40	10–15	0.2
Frosch	65	23.7	
Kober	....	80	
Rosenau	6–17	35	10
Spatta	33	40	25
Whipple	30		

It is important to note the extremely high values assigned to contact in true typhoid which the laity considers a non-contagious one. It is in reality a communicable disease which is conveyed from individual to individual only less often than the so-called contagious diseases, because of the manner in which the organism leaves the body of the diseased. The fact that the organism is given off with the urine and feces considerably lessens the likelihood of its reaching the hands or food of man. There is, consequently, much less danger of being infected by these means than in the case of those diseases whose germs leave the body in the secretions of the nose and mouth.

Gay mentions the following routes by which the typhoid germs may be transferred:

1. Fingers, or utensils, to mouth. (This is the route of direct contact.)
2. Fingers, to food, to mouth.
3. Fomites, fingers, food, to mouth.
4. Flies, food, to mouth.
5. Fomites, flies, food, to mouth.
6. Water to mouth.
7. Water, food, to mouth.
8. Soil, water, or food, to mouth.

Contact.—The disease may be spread by the physician, nurse, and other individuals who care for the ill. This may readily be accomplished by infected fingers, thermometers, tongue depressors, cups, spoons, contaminated towels or clothing. That this occurs is borne out by the fact that physicians and nurses are more likely to contract the disease than others. If often spreads rather rapidly in rural districts where neighbors take turns caring for the ill. This, as pointed out by Vaughan, was the main method by which the disease spread during the Spanish-American War. "Before us every morning regiments were drawn up and so many men detailed from the ranks to serve in the hospitals as orderlies for the day. We followed these men to the hospitals and saw them handling bedpans in their awkward

ignorant way, often soiling their hands, as well as the bedding, floors, and ground. At noon they went to lunch, most of them without washing their hands, to say nothing of disinfecting them, handling their food and passing it to their comrades. A like demonstration was repeated at supper. The next day a repetition of this cycle was repeated." Such procedures caused 20,000 American soldiers to contract the disease, which clearly indicates that an inexcusable lack of cleanliness, resulting either from carelessness or ignorance was prevalent. Contact cases are said to be especially severe. This may be due to the fact that the virulence of the microörganisms has not had any opportunity to decrease outside of the body, as is the case when the individual becomes infected indirectly.

Flies.—It had been suggested on several occasions that the common fly was probably an agent in the spread of typhoid, yet this was not generally understood until the report of the Typhoid Commission appeared in 1898. In this it was shown that flies swarmed over infected matter, becoming soiled with it, and then visited and fed on the soldiers' food. Lime was sprinkled over the latrines, and a few minutes later flies, with their feet whitened with it, were walking over the food. It was further shown that individuals who ate in screened rooms were less likely to contract the disease than were others. Later, Hamilton completed the evidence against the fly by obtaining from its body typhoid germs. It is now known that flies may convey typhoid germs either on their bodies or in their digestive systems, as the typhoid bacilli may pass the alimentary tract of flies without being reduced in virulence. The part played by flies varies widely. It is of small importance in well-sewered cities but often of first magnitude in rural districts. Even aside from the spread of typhoid fever, the fly is a dirty unmitigated pest which should be destroyed.

Milk.—Milk may not only convey the organisms from individual to individual, but it offers a suitable medium for their multiplication. Quite extensive outbreaks of typhoid have occurred in both England and America as a result of infected milk. In 1908 an epidemic occurred in Boston in which there were 410 cases reported. All were traced to the milk which was believed to have been infected by an individual tasting it. Milk-bone epidemics have certain characteristics which set them off rather sharply from those having other origins.

1. The disease follows the route of the milk man. It is at times possible to plot his route from the cases of typhoid.

2. The better class suffers more than do others, as they use more fresh milk.

3. Those who drink milk are chiefly affected; consequently, there is a greater proportion of cases among women and children.

4. The outbreak is explosive in nature, and there is often more than one case in the same household.

5. The incubation period may be shortened, probably because of the large quantity of infected milk consumed.

6. The disease is usually milder than when it comes from contact or water. This is probably because growth in milk decreases the virulence of the bacilli.

The remedies against milk-borne typhoid are: (1) exclude, so far as possible, all typhoid carriers and mild cases from the dairy; and (2) properly pasteurize all milk in the final sealed containers.

Water.—It has already been shown that water plays a prominent part in the spread of typhoid (page 237). In the past the reduction of typhoid has been primarily a result of improved water supplies. In rural districts, however, much still remains to be done in this regard. Water-borne typhoid can be attacked by carefully guarding domestic water supplies against human sewage. This becomes more difficult as the population increases; consequently, it is becoming more necessary each year that water be purified, or treated in some manner before it is used.

Food.—Many foods have been incriminated as carriers of typhoid. The ones which are the most dangerous are those that are eaten raw, especially if they have been produced in sewage-polluted soil or water. Lettuce, radishes, celery, and water-cress have all conributed their quota. When once infected ordinary washing is not sufficient to free them from the typhoid germ.

Oysters, mussels, and shell-fish, if grown in polluted waters and eaten raw, may cause typhoid fever. During 1924–25 there was a widespread epidemic caused by infected oysters. This involved at least 1500 cases and over one hundred deaths. The outbreak was traced to oysters produced in West Sayville, New York, and it was proved that although the typhoid bacilli do not multiply in oysters they may survive in them for several weeks. Inasmuch as oysters are occasionally produced and often fattened in sewage-polluted water there is only one safe procedure; namely, to eat only well-cooked oysters.

The seasonal prevalence of typhoid often gives an index of the possible cause. Fly-borne typhoid occurs only in the warmer

months of the year; water-borne usually in the fall or spring; typhoid due to vegetables and fruits correspond with the season in which these are most used; where as milk outbreaks may occur at any season of the year.

Carriers.—Typhoid fever is a septicemia. The germs pass from the intestines into the blood stream and in this manner visit every organ of the body. It is, thus, not surprising to find that they occasionally locate in some part of the body and later become a menace to the host and especially to the community. The bacilli disappear from the blood several days before the temperature of the body becomes normal, but even in favorable cases they remain in certain parts—bone marrow, gall bladder, and intestines—for several days after the temperature is normal. Occasionally some of them remain in these organs for days, weeks, or even years; and as a consequence the patients become bacilli carriers. The germs which have located in the bone marrow may later cause diseases of the bones; those in the gall bladder may give rise to gall stones, for typhoid bacilli have frequently been obtained in pure cultures from gall stones. Such bacilli carriers are not as great a menace to themselves as they are to the community. The gall bladder serves as a reservoir and a point for multiplication, and from here the organisms are poured into the intestines and appear in the feces.

Typhoid carriers are usually divided into three groups:

Group I.—Precocious or incubation carriers. These are individuals who have ingested the bacilli and are eliminating them before the symptoms of the disease have appeared. There are some individuals who excrete the germs before they themselves develop the disease; usually this is only for a few days, although there are cases on record in which this has occurred for several weeks before the disease has manifested itself.

Group II.—Persons who have recovered from the disease but continue to carry the bacilli. These are of two classes: (1) convalescent carriers—persons who eliminate the bacteria up to three months after recovery; and (2) chronic carriers—persons who eliminate the bacilli for longer periods than three months.

Group III.—Healthy or paradoxical carriers. These are persons who eliminate the bacilli, but have no history of having had the disease.

After a careful study of all available data, Gay concluded that of all the individuals who recover from typhoid fever from 4 to 5 per cent become chronic carriers. From this he concludes that

in the United States about 7,500 carriers are annually added to the number already in existence. The annual reduction in typhoid which has occurred during recent years has also resulted in a corresponding decrease in carriers. Many authorities believe that there are approximately three healthy carriers to every 1,000 persons in this country. This means that the United States still has an army of about 300,000 carriers, and Gay considers it conservative to attribute one-fourth of all cases of typhoid fever to carriers.

The actual danger of a given carrier varies with his occupation. Lumber-men, miners, stenographers, and the like may be of little danger, but cooks, milkmen, and all individuals engaged in the production, handling, and vending of food, especially that which is eaten raw, are a great menace. The examination of about 30,000 food handlers in the army during the World War revealed the fact that less than 0.1 per cent of the healthy males were carriers. The danger in civilian life is greater than these figures indicate, for there are about five times as many female carriers as male. Most carriers are adults.

A remarkable instance of a typhoid carrier was the one investigated by Soper and known in history as "Typhoid Mary." Even when detected she refused to coöperate with the investigators in furnishing information concerning her past history, but definite information was obtained back as far as the year 1901. At that time "Typhoid Mary," whose real name was Mary Mallon, was employed as a cook. A visitor developed typhoid ten days after visiting the family in which she worked. In 1902 she obtained another position. Two weeks later the laundress was taken ill with typhoid. Soon seven members of the household were ill with it. In 1904 she went to a family in Long Island; within three weeks after her arrival four servants were ill with the disease. In 1906 she went to another family. Soon six out of the eleven inmates were ill with the disease. Here for the first time she was suspected but was not proven to be a typhoid carrier, until she had entered another family and infected the laundress. Mary was tested and found to be excreting typhoid germs in great numbers. Soper writes: "The implication was plain; the cook was virtually a living culture tube in which the germs of typhoid multiplied and from which they escaped in the movements from her bowels. When at toilet her hands became soiled, perhaps unconsciously and invisibly, so when she prepared a meal, the germs were washed and rubbed from her fingers into

the food. No housekeeper ever gave me to understand that Mary was a particularly clean cook." She was detained for some time by the New York Department of Health, but later paroled on the promise that she would not take employment as a cook. She soon broke her parole and nothing was heard of her for five years. An outbreak of typhoid fever in the Sloane Hospital for Women revealed the fact that "Typhoid Mary," under the name of Mrs. Brown, was doing the cooking. Since then she has been virtually a prisoner. This girl was proven the sole cause of 51 cases of typhoid in ten outbreaks. Inasmuch as her whole history is not known she may have been the cause of many others.

Another destructive outbreak in Hanford, California, was investigated by Sawyer. It resulted in 93 cases of typhoid fever with a number of deaths. The vehicle of the infection was a large pan of spaghetti prepared by a carrier who gave no history of having had typhoid. The spaghetti was baked after it had been infected, but laboratory test showed that the baking incubated the bacteria instead of destroying them.

Many means have been used in attempts to cure typhoid carriers. These have met with only partial success. Consequently, in most cases when typhoid carriers are detected, they are made to promise that they will not engage in work where they are required to handle food for others, and that they will keep the Health Department of the city informed of their presence.

Typhoid Vaccination.—Serums have been prepared as curative agents in typhoid fever, but the efficiency of these has not yet been proven. On the other hand, very efficient preventive vaccines have been prepared. The value of these have been fully established in both civil and military life. The results in military life are especially convincing, as the following table shows:

TYPHOID IN THE U. S. ARMY (CASES PER 100,000 MEN)

	Cases
1901 No vaccination	674
1905 No vaccination	314
1909 Voluntary vaccination	335
1910 Voluntary vaccination	243
1911 One-half Compulsory	85
1912 Compulsory	31
1914 Compulsory	7.5
1915	2

In 1898 during the Spanish-American War, there were 4,422 cases of typhoid and 248 deaths among 10,759 men. In 1911

there were 12,801 vaccinated men held in San Antonio, Texas, where typhoid fever was prevalent among the civilians, but only one case developed among this body of men. In the army of the Spanish-American War there was one case of typhoid for every seven men, and one death for every 71 soldiers, whereas during the World War the U. S. Army had only one case for every 3,756 soldiers and one death for every 25,641 soldiers.

Prevention.—Typhoid fever can be prevented in two ways: first by destroying the typhoid bacillus; and second, by preventing the bacillus from reaching the body of other human beings. It leaves primarily in the feces and urine; consequently, these should be disposed of under all conditions, so that they do not reach food and water. This requires properly constructed sewer systems, and where toilets must be used they must be properly screened. Water should come from deep wells, springs, streams, or reservoirs which are protected from human pollution. Milk should be pasteurized, and food likely to convey the disease cooked. Every individual should learn and practice general rules of hygiene, such as the washing of the hands before meals and always after visiting the toilet. Every individual should treat himself as a possible carrier, insofar as personal cleanliness is concerned.

Individuals ill of typhoid, and also the recent convalescent, must be considered as typhoid incubators and special precaution taken to disinfect their excreta. Typhoid differs from most communicable diseases in that it yields to sanitary measures. It is in reality a filth disease, and absolute cleanliness insures the absence of typhoid fever. Furthermore, every case of typhoid prevented means the elimination of a future carrier; hence, as the number of active cases decreases the number of carriers is reduced and the problem becomes less acute. Wherever individuals must associate with persons ill of the disease, active carriers, or live where food and water are polluted, the only safeguard is vaccination. This is not advocated for the population as a whole, for: (1) the disease in times of peace and in the absence of epidemics can be controlled by sanitary measures; and (2) immunity by typhoid vaccination is comparatively short and must be repeated every few years.

CHAPTER XL

INFECTIONS COMMON TO MAN AND LOWER ANIMALS

Man differs from the lower animals in many respects. An intrinsic difference between the two is to be found in the relationship to bacteria. Many microörganisms attack only man, and invade only human tissues; while others attack only the lower animals. However, there is a limited number that attacks both man and the lower animals. Typhoid, cholera, measles, and many others are peculiar to man. Canine distemper, chicken, and hog cholera are limited to the lower animals. Anthrax, glanders, rabies, and bovine tuberculosis are diseases which have been known for ages as common to man and his domestic animals, and as research progresses new diseases and new relationships of old diseases are being discovered. Tetanus and tuberculosis, both diseases common to man and the lower animals, have already been considered. In this chapter consideration will be given to anthrax, glanders, malta fever, foot-and-mouth disease, and tularemia; and in the subsequent chapters to plague, and rabies. Most of these are primarily diseases of the lower animals and only secondarily of man; consequently, it is evident that their eradication in man rests upon their control in the lower animals.

Anthrax.—Anthrax is primarily a disease of the herbivora. Cattle and sheep are especially attacked, and frequently horses, hogs, and goats. In cattle it is a rapidly developing, very contagious, and highly fatal disease. The preliminary symptoms are rather indefinite; consequently, it can usually be recognized mainly by its very rapid development, and the appearance of the internal organs after the death of the animal. Cattle die of it in two to five days. The blood is dark, and thick, and the affected animals have an enlarged spleen. Because of this last characteristic the disease is sometimes spoken of as spleenic fever.

Man is susceptible to anthrax and can contract it through association with diseased animals, or through the use of products from infected animals. The organism may enter the body through feeding or inhalation. A break in the continuity of the skin or mucous membrane invites infection when the organism is present.

When it enters the body of man through the alimentary tract in infected food or water, the resulting disease is highly fatal. Wool pickers and workers in horse hair and other raw products, which may come from infected animals, contract the pneumonia type of the disease. This is known as wool sorters' disease and is frequently fatal. The immunity of man is fairly high, and when it enters through an abrasion the result is usually local. This may occur on the hands of butchers, veterinarians and others. It often occurs on the shoulders and backs of individuals who carry the raw hides onto ships in some South American ports.

Historical records contain almost countless instances in which references are made to the terrible havoc that this disease wrought in the sheep, cattle, and horse industries of the past ages. Kircher, a Jesuit monk, describes an outbreak which occurred among cattle in 1617 but later spread to man and claimed 60,000 victims among the latter. It still occurs in most sheep- and cattle-raising districts, but is more prevalent in some regions than in others. It is especially prevalent in Germany, France, Austria-Hungary, and Russia. It is stated that 72,000 horses died of this disease in Russia during 1864. In England and America the disease is relatively infrequent. The number of human cases varies with the number among the lower animals. Consequently, during the periods and in those districts where it is prevalent among domestic animals mankind suffers most. Bellon reported between 1909 and 1924 205 cases that occurred in Marseilles among industrial workers. Twelve per cent of these were fatal. There are about 130 cases annually in the United States. These occur among laboratory workers, veterinarians, meat inspectors, farmers, cattlemen, butchers, and especially workers with wool, hair, and hides. There was an increase in the number of cases during the World War, 23 cases being reported from New York alone. These came mostly from the use of infected brushes, especially the shaving brush.

Anthrax was the first pathogen to be seen under the microscope, as well as the first pathogen to be obtained in pure culture. It likewise, was the first disease to be transmitted by inoculation to susceptible animals, and was the disease which Pasteur so dramatically demonstrated could be prevented by a vaccine. Bacteriological work on this disease was to transform it from one which threatened to mercilessly and quickly devastate the entire cattle and sheep industry and before which man stood helpless

and amazed into one which man can fully control. The net gain in dollars has run into the billions.

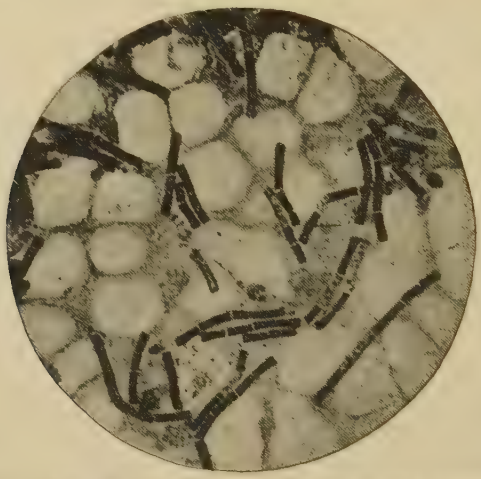


FIG. 122.—Bacillus anthracis. (*Fränkel and Pfeiffer.*)

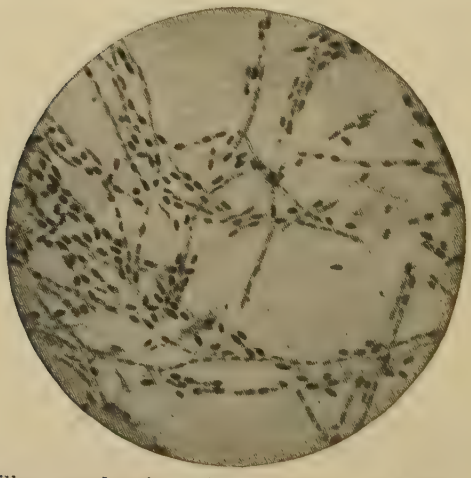


FIG. 123.—Bacillus anthracis with spores. × 1,000. (*Fränkel and Pfeiffer.*)

The anthrax organism is a slender cylindrical non-motile rod with squarely-cut ends. It is from 1 to 3 microns in diameter and 5 to 10 microns in length. On artificial media and in the presence

of oxygen it forms spores which are very resistant. The spores do not form in blood until it is exposed to air; consequently, the bodies of animals which have died of the disease should be destroyed without letting their blood reach the soil. The spores withstand drying, strong germicides, and high temperatures to a much higher degree than most pathogens. In this respect it is similar to the botulinus and tetanus organisms. The anthrax bacilli can survive for years in soil, and upon hides which have passed through the tanning process; consequently, when the organisms once enter the soil of pastures they persist, although there is no evidence that they multiply in the soil. It was therefore once dangerous to graze cattle on pastures where the anthrax bacteria were present. Today, however, as a result of Pasteur's work, properly vaccinated cattle can be grazed on such pastures with impunity.

The prevention of anthrax in man rests primarily upon its eradication in domestic animals, and secondarily upon the disinfection of all products obtained from infected animals. As already mentioned, the bodies of animals that die of the disease should be destroyed without spilling their blood on the soil. Stables in which diseased animals have been kept should be thoroughly disinfected. Animals to be housed in such buildings or grazed on infected soil must first be vaccinated. Individuals dealing with infected material should use constant care not to infect themselves. This is accomplished by the wearing of rubber gloves in postmortems, and the disinfecting of hides, hair, and wool before they are worked into finished products for the use of man. Proper ventilation for the carrying away dust where these products are being handled goes far in the protection of the worker. The refuse from tanneries, wollen mills, and the like should be properly disposed of to prevent soil or water infection.

Glanders.—Glanders is a communicable disease of horses, mules, and asses. Goats, rabbits, and dogs may contract it in the natural manner. It is readily inoculated into the ordinary laboratory animals, with the exception of the white mouse which is said to be immune. Cattle are immune. Man is quite susceptible and may contract it either from his fellowmen or from the horse, which is more usually the case. It has a high mortality in both man and the horse, but it is said to be most fatal in man when contracted from other human beings. Robins reported that of the 156 cases of glanders in human beings which he investigated, the percentage of recovery was found to be less

than 6 per cent. The duration of the disease averaged fourteen and a half months with a variation of from six weeks to fifteen years.

The infection enters the body through either abrasions in the skin or the mucous membrane. It is characterized by the formation of inflammatory nodules in the nose or skin. The first form is known as glanders; the second, farcy. The nodules break down leaving a crater-like ulcer. Glanders may occur in either the acute or chronic form. Gaiger, who was in the Indian Civil

FIG. 124.—Condemned for glanders. (*Courtesy of Dr. Frederick.*)

Veterinary Department, gives his experience with the disease which he contracted from an infected Arabian pony. The symptoms appeared March 4, 1911, and by June 25, 1913, the last lesion had healed. In the treatment he underwent no less than 45 operations, of which 27 were done under general anaesthetics. One of the major operations was for the amputation of the right arm.

The cause of glanders, *Pfeifferella mallei*, is a rod shaped microörganism with rounded ends. It measures from 0.5 to 0.7 micron, is non-motile and does not form spores. It stains feebly with watery solutions of the basic analine dyes, but deeply with Löffler's methylene blue or carbol fuchsin. With the last two it assumes

a striated appearance. It grows slowly on laboratory media. Its optimum temperature is that of the human body. It is not very resistant to heat or antiseptics. However, it can withstand drying for ten days and may survive for one or two months in water. Due to this property it is often spread in horses' drinking troughs. There is no evidence that it naturally multiplies outside the body of animals. Its prevention in man rests upon its

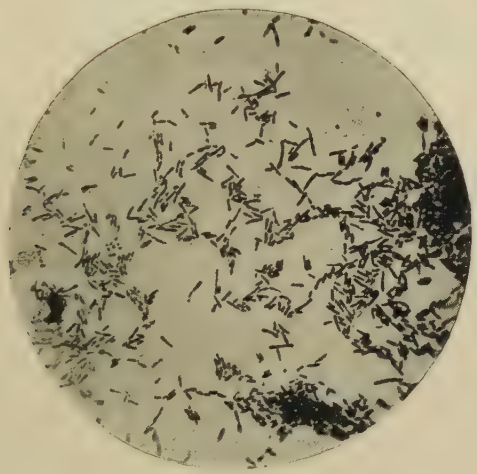


FIG. 125.—Pfeifferella mallei. (*Fränkel and Pfeiffer.*)

prevention in the horse. The use of the automobile has very materially reduced the number of human cases.

Malta Fever.—A disease known as Malta fever has existed on the Island of Malta and elsewhere about the Mediterranean for years. More recently it has appeared in several other countries, including the United States. It is primarily a goat disease and is contracted by man from this animal, mainly through the use of infected milk, and occasionally through wounds.

It is characterized in man by headache, sweating, pains in the joints, arthritis, constipation, enlarged spleen, and irregular fever, and may persist for weeks or even months. The mortality is less than 2 per cent.

Bruce discovered the cause to be a microörganism, now designated as *Bacterium melitense*. He did not explain the mode of infection. This was in doubt until 1905, when 65 goats, all

apparently in healthy condition, were shipped from Malta to the United States. The raw milk was drunk during the passage by the captain and a number of the crew with the result that many of them contracted Malta fever. On arriving in America, *Bacterium melitense* was found in the milk of several of the goats, and the blood of 32 of them gave a positive agglutination test with the organism.

The organism which causes Malta fever is a minute bacillus and measures from 0.2 to 0.4 micron and was formerly described as a coccus (*Micrococcus melitense*). It is non-motile, does not

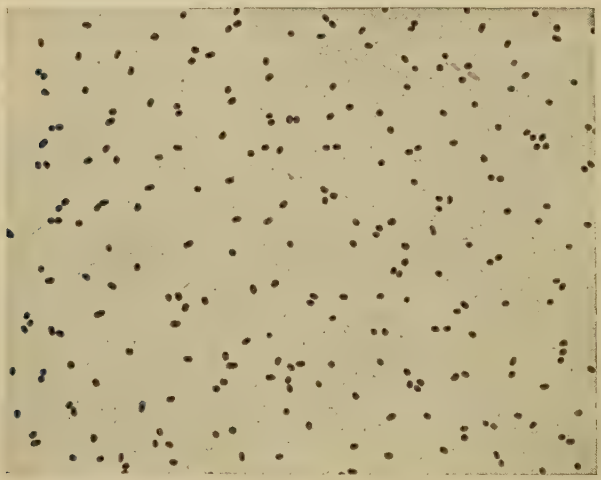


Fig. 126.—Bacterium melitense. (*From Ford, A Text-book of Bacteriology.*)

form spores, and stains well with the ordinary stains. The organism withstands cold well, and is said to be able to survive in dust for many months. The *Bact. melitense* is so like the *Bact. abortum*, which causes disease in cattle and hogs, that the two cannot be distinguished by ordinary laboratory methods. Some authorities believe them to be different strains of the same organism. If this be true the abortus strain is much less virulent for man than is the Malta strain, as the former is very prevalent in cows' milk, and is either not highly virulent or produces a very mild affliction in man. A number of human cases of *Bact. abortum* infection have been reported in the United States, one of which

was fatal. Strains from the hog apparently are more infectious to man than are those from cattle. The control of these diseases in man rests upon their eradication in goats, cattle, and hogs. Closer coöperation between the veterinarian and the public health worker can do much. The general use of pasteurized milk would practically eradicate these diseases in man.

Foot-and-mouth Disease.—Foot-and-mouth disease is an acute, highly communicable disease attacking chiefly the cloven-hoofed animals. Among human beings it occurs most frequently in children and usually results from the use of raw milk from diseased animals. However, it can result from the transfer of other infectious secretions. In cattle it is characterized by lameness due to lesions about the hoof, small vesicles in the mouth, loss of appetite, great emaciation, and marked diminution in the quantity of milk secreted. The mortality is low, but the economic loss is high due to impaired nutrition of the animal and the reduction in the milk flow. There is apparently no lasting immunity; consequently, once introduced into the herd the influence is long manifest. In human beings the disease is characterized by fever, sensation of heat and dryness in the mouth followed by the eruption of vesicles distributed over the lips, gums, edge of tongue, more rarely on the fingers and seldom on the toes. The disease is usually quite mild, although its results may occasionally be fatal in sickly or very young children.

Epidemics of the disease have occurred in various parts of the world and is endemic in many countries at all times. It has been introduced into the United States on eight different occasions: into the Eastern States in 1870; into Massachusetts in 1880 and 1884; into New England in 1902; into Detroit in 1908; into Chicago in 1914; into California in 1924; and into Texas in 1925. Each time it has been stamped out by the quick, systematic, cooperative work of local and federal officers. Often this has necessitated the destruction of whole herds of infected cattle with great economic losses. Consequently, quarantine is rigorously enforced. Infected manure, hay, utensils, drinking troughs, railway cars, animal markets, and barnyards; all may contribute in the spread of the virus.

The virus will pass the finest porcelain filters, as was shown by Löffler and Frosch in 1898. In April, 1924, Frosch demonstrated before the Microbiological Society of Berlin an exceedingly small bacterium which he had succeeded in photographing with the aid of the ultra-violet ray. He had cultivated this organism on

both fluid and solid media, claimed to have successfully infected guinea pigs, and caused mild symptoms in cows. He has proposed calling the organism *Löfflería nevermanni*. A British and also a German commission so far have failed to confirm these findings.

Tularemia. McCoy in 1911 described a plague-like disease which occurred among the ground squirrels of Tulare County, California. The following year he and Chapin succeeded in obtaining from diseased squirrels and growing on an egg medium a very small rod, 0.3 to 0.7 micron in length and often capsulated. The organism is found in large numbers in the spleen of the animals that have died of the disease. To this organism they gave the name of *Bacterium tularense*. It is killed at 56° to 58° C. in ten minutes, but withstands drying and cold for comparatively long periods. It is readily killed by formaldehyde, cresol, and the usual germicides.

It causes a disease in wild rabbits, ground squirrels, and other rodents, and in the case of the first named often brings about fatal results. It also attacks man causing a typhoid-like disease. Human cases and rodent infection have been reported from a number of states, but the greater number has come from the western part of the United States.

The disease may be transmitted from rodents to man by the woodtick and by the deer-fly commonly found on horses in Utah and the adjoining states during the months of May, June, and July. Insects, however, are not essential to its spread, as a number of laboratory workers have been infected possibly in some cases through the undamaged skin. It can also be transmitted by eating improperly cooked tissues of diseased animals. This occurred in four cases in Lee County, Virginia, three of which were fatal. A number of cases have been reported in which the primary seat of infection was the eye into which the infectious material had been mechanically transferred from rabbits or ticks. It has been suggested by Parker and Spencer that infection may take place by inhalation where laboratory workers are exposed to animals with extensive pulmonary involvement. There is no evidence that it tends to spread from man to man.

It has been shown that *Bacterium tularense* can be hereditarily transmitted in the intermediate host, the woodtick, from parent insect to eggs, nymphs, and larva. This makes the difficulty in its control greater than if infection came to the tick only by the biting of infected animals.

The prevention rests upon the care exercised in the handling and use of infected animals. Extreme care should be taken when dealing with susceptible animals in districts in which infection is prevalent. Rubber gloves should be worn in the dressing of wild rabbits, and every precaution taken to see that the animals to be eaten are not diseased, and even if they are not infected to cook the meat thoroughly.

CHAPTER XLI

SOME INSECT-BORNE DISEASES

It has been observed for centuries that certain so-called contagious diseases are peculiar in their geographical distribution, seasonal prevalence, and erratic method of spreading. Numerous theories were evolved to account for this, but the true explanation did not come until the brilliant work of Smith, Manson, Finlay, Ross, and a score of others had been carried on in connection with what we now recognize as insect-borne diseases. The beginning of this new period in preventive medicine dates back to 1893 when Theobald Smith demonstrated that Texas fever, a disease of cattle, is transmitted only by the bite of a tick. Since then, many other diseases have been shown to be transmitted in a similar manner. These discoveries have placed in the hands of health workers means of subduing and in some cases of eradicating plagues which in the past had devastated the most fertile regions of the world and claimed millions of victims.

Classes of Insect-borne Diseases.—There are two classes of insect-borne diseases: namely, the biological, and the mechanical. The biologically-transmitted diseases are directly dependent upon some biting or sucking insect for transmission. It is in the body of this insect that certain definite stages of the infective agent's life cycle are passed. So far as known, these infective agents have no other means of transmission. Usually the transmitting agent is limited to a specific species, or to a very limited number of nearly related species of insects, but whatever the case may be these insects when they are once infected remain so throughout their lives. The infecting agent, so far as is known, belongs to the animal kingdom. It is evident that if the infecting agent passes a part of its life cycle in the insect, there must elapse a certain incubation period between the time when the insect becomes infected and the time when it is able to transmit the infection. This period of non-infectivity is known as the extrinsic period of incubation and represents the time necessary for the infective agent to complete its sexual cycle in the insect and reach the appropriate point of exit. Usually the insect

becomes infected by feeding on some infected animal. In a few diseases, like Texas fever, Rocky Mountain spotted fever, and African relapsing fever, hereditary infection may occur. Some of the more common biologically-transmitted diseases are:

Disease	Cause	Insect
Malaria	*Plasmodium malariæ*	Anopheles mosquito
Rocky Mountain spotted fever	*Dermacentroxenus rickettsi*	Tick
Texas fever	*Babesia bigemina*	Tick
Typhus fever	*Rickettsia prowazeki*	Louse
Yellow fever	A filterable virus	Aëdes mosquito

The mechanically-conveyed diseases are incidentally transmitted by insects. The insects act as would an infected needle. They are not essential to the propagation of the virus. The infecting agent may be upon or within the body of the insect and is usually bacterial in nature. There is no incubation period in the body of the insect, and the transmission is not necessarily limited to a single species of insects. The more common members of this group of diseases are:

Disease	Cause	Insect
Cholera	Cholera bacilli	Flies
Dysentery	Bacteria and ameba	Flies
Plague	*Bacillus pestis*	Fleas
Typhoid	Typhoid bacilli	Flies
Tularemia	*Bacterium tularense*	Tick, deerflies

Characteristics of Insect-borne Diseases.—Although insect-borne diseases may occur in epidemics, they are never world-wide in their distribution, for it is evident that they are limited to those districts in which the insect and the infecting agent prevail. This gives these diseases a seasonal variation which is dependent upon the prevalence of the insect. They also vary with rainfall and temperature, for these determine the distribution of the insect. However, the temperature governs not only the occurrence of the insect, but in some of the biologically-transmitted diseases it also determines whether or not the infecting agent can pass its life cycle in the body of the insect. It is known that insects do not migrate great distances of their own accord, but these are

occasionally mechanically transferred long distances by modern vehicles of transportation. Consequently, the infecting agent is usually distributed to various quarters of the globe by man where, if appropriate insects occur, the disease may become epidemic. We shall now consider in some detail malaria as a type of the biologically-transmitted disease, and plague, as a type of the mechanically-transmitted disease.

MALARIA

Historical.—For ages malaria has held a large proportion of the population of the globe in its bondage. There appear in the writings of Aristophanes (about 422 B. C.) clear descriptions of the disease, and Hippocrates states that men who drink marsh waters get enlarged spleens, and those who live in low, marshy districts are neither tall nor well built, but dark-colored and wanting in courage and endurance. This reflects the belief that was held even to a comparatively modern period. It is reported that Empedocles (about 550 B. C.) subdued the disease in a city in Sicily by draining its swamps. The first step in its conquest, however, probably came about 1660 when Countess d' El Cinchon, wife of the Viceroy of Peru, was cured of malaria by a concoction made from the bark of a native tree. She spread this knowledge among Europeans, and for two hundred years there was a difference of opinion concerning the therapeutic value of this agent. In some cases it wrought miraculous cures, while in other cases it was without effect. Today we understand that what seemed failures which occurred in many cases were due to the fact that fevers other than malaria were being treated. In 1820 Pelletier and Caventou extracted from the cinchona bark the active constituent, since known as quinine; but it was not until 1880 that Laveran, a French army surgeon, announced the discovery of the malaria parasite in the human red corpuscles of persons ill with the disease. But even this discovery was not sufficient to make possible the control of the disease. This did not take place until 1895 when Ross proved that the anopheles mosquito is responsible for the spread of the disease, and today we know that the control of the disease rests upon the destruction of the mosquito and the use of quinine.

Prevalence.—Malaria has played a leading rôle in the history of the nations. It is held by Jones and others that the fall of Greece, and particularly of Rome, was due in a large measure to

malaria, which was introduced into Rome with the slaves from Africa. The colonization of tropical Africa and Central America has been controlled by malaria. It has been estimated that it is even at the present time the direct or indirect cause of over one-half of the entire mortality of the human race. Even today there is no other disease which compares with it in importance. Sir Ronald Ross holds that India alone loses over one million persons annually from this disease, a greater number of deaths than resulted during the first two years of the World War. Malaria claims its victims by the thousands in Africa, Southern Europe, South, and Central America. Even the southern part of our own country loses approximately twelve thousand persons annually from this disease. Dr. Howard estimates that there are annually three million cases in the United States and it is believed by some that malaria is one of the most potent reasons why the South is backward, for malaria not only kills, but it also, devitalizes. It has been estimated that malaria, in one form or another, places an annual tax of $14 an acre on all tillable land in Georgia and $4 an acre on all land in Louisiana. Dr. Howard also makes the astonishing but well-founded statement that the annual financial loss to the United States from malaria is not less than $100,000,000.

FIG. 127.—Anopheles mosquitoes. (*Howard.*)

Malarial Parasites.—The malarial parasite belongs to the animal kingdom. Three species are now recognized, which are associated with three distinct types of the disease: *Plasmodium vivax*, causing tertian fever, which is comparatively mild and rare; *Plasmodium malariae*, causing quartan malaria, which is common and likely to relapse; and *Plasmodium falciparum* causing aestivo-autumnal malaria, which is most malignant and pernicious in tropical places. These organisms pass their sexual life in the body of the mosquito, and their asexual life in the body of man. The sporozites are present in the saliva of infectous mosquitoes and are injected into the

human being when the insect bites and withdraws blood. On entering the blood the sporozoites grow at the expense of the red corpuscles and possibly also obtain nutrients from the plasma. In

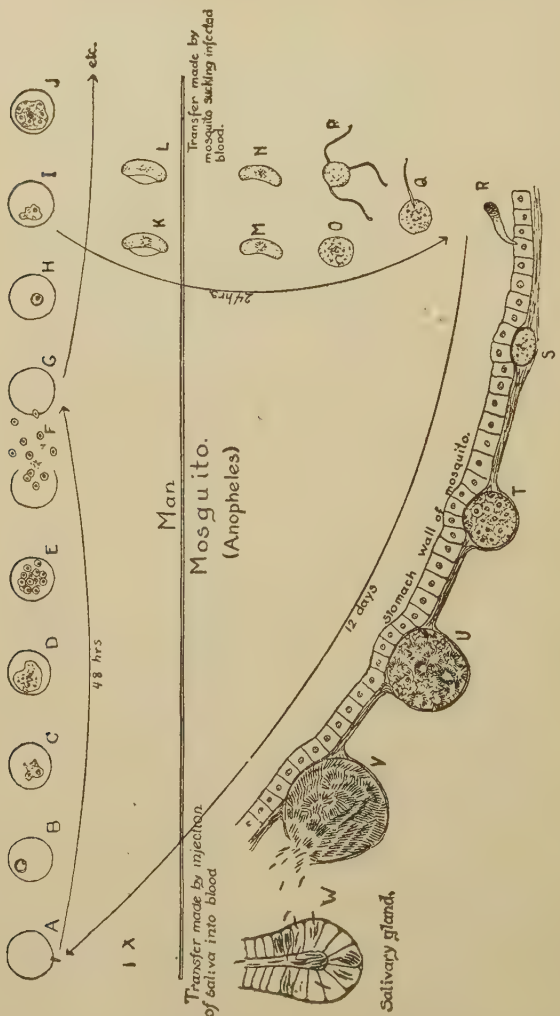


Fig. 128.—Life history of malarial parasite. (*After Chandler.*)

its youngest recognizable stage, the malarial parasite appears to be attached to the corpuscle as a small, glassy, oval, signet-ring-like body with ameboid movements. It proceeds to burrow slowly

into the substance of the corpuscle, and increases in size at the latter's expense. On attaining its maximum development, which varies in size with the species, the parasite undergoes a segmentation of its cell substance, giving rise first to a rosette or mulberry-like body within the corpuscle and leading eventually to the formation of small, round spores, or merozoites. These are liberated by the ultimate disintegration of the corpuscles. On being set free, they fasten themselves to new red corpuscles and begin their cycle of development again.

The cycle from one generation to the next varies with the species of the malarial parasite and may be as short as twenty-four hours. The bursting of the corpuscles and the release of the poisonous products coincide with the characteristic chills and fever of malaria. This rapid multiplication may give rise to enormous numbers of the parasites in the blood. The actual quantity in the human body in a case of severe estivo-autumnal malaria has been estimated at 600 cc., or over one pint. This is approximately 3,000,000,000,000 malarial parasites, a number fifty times greater than the seconds which have elapsed since the birth of Christ! As long as the blood of the host is a suitable environment, they continue to multiply in regular manner; but when conditions become unfavorable due to the production of anti-bodies in the blood of the patient, they develop special sexual forms, or gametocytes, male and female, in the form of sausage-shaped crescents, and remain in this form until sucked up by the mosquito. If the temperature is right these gametocytes pass through a complex sexual phase in the digestive tract of the mosquito. The female gametocyte is fertilized by the male, elongates, and becomes quite like a little worm. This finds its way between the inner and outer lining of the stomach and here develops spores. At times a single capsule may contain 10,000 spores, and there may be 500 capsules on one mosquito's stomach. In about twelve days, depending upon the temperature, after the infected blood has been taken by the mosquito, the capsules are mature and rupture, and the spores find their way into the saliva of the mosquito and from here into the blood of the human host when bitten by the mosquito.

Method of Spread.—Malaria is spread solely by the bite of the anopheles mosquito. Over one hundred species of this insect have been described, but less than half of these have been proved to harbor the malarial parasite. It forages out-of-doors in the twilight. This is often taken advantage of by individuals living

in malarial districts. They may bite at any hour of the day indoors; consequently, careful screening is essential in malarial districts.

The anopheles mosquito breeds in almost any standing water, provided it contains microscopic organisms on which it can feed. The eggs are deposited on the surface of the water and float separately on their sides. In a day or two these hatch into larvae or "wiggle tails." These ordinarily rest and feed just below and horizontal with the surface of the water. In about a week they develop into curiously shaped creatures known as pupæ. These pupæ have no mouths; consequently, they do not feed but are air-breathing animals and remain near the surface of the water. In two or three days there emerges from these pupæ the adult mosquitoes, the imagoes.

Prevention.—The control of malaria rests upon either the destruction of the malaria parasites in man by the use of quinine, or the destruction of the mosquitoes. The latter method is most effective, and depends first of all, upon the destruction of the natural breeding places of the mosquitoes. The manner in which this method is to be carried out varies with the locality. At times it means the draining of swamps, at other times, the filling in of low places. Where neither of these methods is feasible the stagnant water may be oiled or at times planted with fish. The fish feed upon the mosquito larva, and under favorable conditions are very effective in cutting down the number of mosquitoes which is all that is essential in the control of malaria as it is not necessary to completely eradicate the mosquito.

PLAGUE

Plague is primarily a disease of the rat and secondarily of man. Often rats die in great hordes during plague epidemics. Plague is generally transferred from rat to rat and from rat to man by the flea. In man it takes three forms: (1) *Bubonic Plague*—This form of the disease is spread by the flea. The bite usually occurs on the legs, and the germs in attempting to spread are caught by the lymph nodes, in this case in the groin. These swell and often suppurate. The swollen glands are known as "buboes" and the disease as the "bubonic plague." The mortality varies, but is usually from 40 to 70 per cent. (2) *Pneumonic Plague*—This form is spread by contact. The lungs are involved and mortality is nearly 100 per cent. (3) *Septicemic Plague*—

In this numerous hemorrhages occur under the skin which turn black. This has given rise to the term "the black death." It is transmitted by the bite of the flea. Although there is a tendency for each form to run true, yet, occasionally, the one form changes to the other and when once changed to the pneumonic the spread is rapid.

Both sacred and profane history abounds in references to plague. Quickly and with a great fury it has often swept time and again over large areas of the civilized world. Tacitus describes the plague days of 68 A. D. in these words: "Houses were filled with dead bodies, and the streets with funerals; neither age nor sex was exempt; slaves and plebians were suddenly taken off, amidst lamentations of their wives and children, who while they assisted the sick or mourned the dead, were seized with the disease, and perishing were buried on the same funeral pyre." When the plague days in Rome (80 A. D.) were at their height the victims in this city of 1,000,000 numbered 10,000 a day. The plague constantly smoldered in the cities of Europe and Asia and periodically burst into a raging demon. The plague of 1348 claimed 25,000,000 victims in Europe, one-fourth of the total population. During this epidemic the population of Rome was reduced to 20,000. In the face of such a devastating plague we are not surprised to find Boccaccio writing concerning the plague days in Florence: "Such was the cruelty of Heaven and perhaps of man, that between March and July following, it is supposed and made pretty certain, that upwards of 100,000 souls perished in the city alone, whereas, before the calamity it was not supposed to have contained so many inhabitants. What magnificent dwellings, what noble palaces were depopulated to the last persons, what families extinct, what riches and vast properties left, and no heir to inherit; what number of both sexes in the prime and vigor of youth—who in the morning Galen, Hippocrates, or Æsculapius himself would have declared in perfect health—after dining heartily with their friends here have supped with their departed friends in the other world."

During the Middle Ages it struck with terrific fury in many parts of Europe, sweeping everything before it, leaving confusion, panic, and death in its trail. And even in more modern times it has been trailed, time and again, across the American continent. Even today it requires constant, careful vigilance to keep it from flaring up in Europe and America. In India it still claims its victims by the millions.

The Cause.—During 1894 Kitasato and Yersin, working independently of each other and almost simultaneously, obtained from the buboes the *Bacillus pestis*. Since then this has been repeatedly done by others, thus establishing the principle that the organism always occurs in the body of the diseased. The evidence that it is the specific cause has been furnished by several laboratory accidents which have resulted in the plague. The organism is a short, thick rod with well-rounded ends varying in length from 1 to 2 microns, and in diameter from 0.5 to 1 micron.

FIG. 129.—The plague bacillus. (*From Ford, A Text-book of Bacteriology.*)

It shows great variation in shape and is prone to produce involution forms, especially when grown on salt agar (agar containing 3 per cent sodium chloride). When properly stained the two poles are deeply colored, whereas the central part remains unstained. It is non-motile and produces no spores although under appropriate conditions a capsule can be demonstrated. It is aërobic and grows best at a temperature of about 30° to 35° C. It is quite resistant to cold but is readily destroyed by heat, drying, and the ordinary disinfectants. The plague bacillus is readily isolated from infected tissue by rubbing the material on the shaved area of a guinea pig. The skin of the guinea pig acts as a differential filter and permits the plague bacilli to

enter but keeps out other organisms. Consequently, the bacillus may be obtained in pure culture from the body of the infected animal.

Method of Spread.—Plague, as already mentioned, is primarily a disease of the rat and secondarily of man. It is also prevalent among some other animals, as for example, the ground squirrel in California, and the marmot in certain parts of Tibet. The transfer of the virus from the rat or other animal to man is made mechanically by the flea. The ingested blood may be regurgitated by the flea into the bite, or more often the virus traverses the alimentary canal of the flea and is deposited with the feces, and this rubbed into the skin by scratching. The pneumonic form is spread by direct contact.

Prevention.—It is impossible to exterminate the flea, as all who have lived in flea-infected districts know; but it is possible to get rid of the plague-infected rats and ground squirrels. A systematic vigorous campaign has been waged for years in California against infected ground squirrels, with the result that the plague is kept within bounds. Campaigns against rodents which keep the plague alive should be vigorously pushed, for in the case of the rat it is estimated that annually it wastes and destroys property in the United States to the value of $167,000,000. Moreover, the rodents are known to convey, in addition to plague, by one means or another the following diseases: acute infection, jaundice, food infections, trichina, tape worm, other parasites, and rat-bite fever.

Rodents may be destroyed by trapping, poisoning, destruction of breeding places, the keeping of dogs or cats which prey upon them, and the building of rat-proof storehouses for grains and other things on which they feed.

CHAPTER XLII

THE FILTERABLE VIRUSES

The work of the last decade of the nineteenth century brought into prominence a group of infecting agents capable of passing through extremely fine filters. Consequently, they were referred to as filterable viruses. In the early years of their discovery none of the viruses had been seen with the microscope; consequently, the term "ultramicroscopic" was used synonymously with filterable. More recent work, however, has developed methods which render a number of the filter-passing viruses visible. Yet, even today there are numerous filterable viruses which remain in the domain of the invisible. Whether their invisibility is due to minute size or composition is not clear, nor is the biological relationship of the various members known; nevertheless, the term filterable virus is a convenient one to use when reference is made to the heterogenous group of filter-passing infecting agents. They are probably corpuscular in makeup and possess the characteristics of living matter. They are considered by some authorities to be living entities, whereas others believe them to be certain forms of self-reproducing enzymes.

History.—The first systematic study of a filterable virus was made by Pasteur on rabies in 1881, but it was not until 1892 when Iwanowski in the course of his investigations of the mosaic disease of tobacco that there was demonstrated filter-passing viruses pathogenic for plants. This Russian investigator found that he could produce mosaic disease in healthy plants by inoculating them with the filtered juice from diseased leaves. Six years later Beijerinck published confirmatory evidence of these findings, and during the same year Löffler and Frosch published their epoch-making studies on foot and mouth disease. These investigators found that they could produce the disease by inoculating animals with the filtrate made from the contents of the vesicles occurring on the mouths of diseased animals. They passed this from animal to animal, each time causing the disease, thus demonstrating that the specific causative agent multiplies

within the body of susceptible animals. Since 1898 many diseases have been shown to be due to filterable viruses, but as yet there is no absolute agreement among authorities as to all the diseases which should be classified as caused by filterable viruses. The following list given by Rivers of the various diseases which have at one time or another been classed as due to filterable virus is suggestive.

DISEASES WHICH AT ONE TIME OR ANOTHER HAVE BEEN CLASSED AS FILTERABLE VIRUS DISEASES

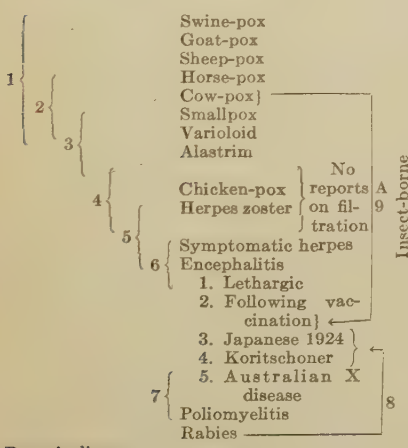
Bacteriophage

Mosaic diseases of plants
(infectious chlorosis)

Sacbrood
Wilt of European nun moth
Wilt of gypsy moth caterpillar
Jaundice of silk worms

Epizoötic of guinea pigs
Hog Cholera
Cattle plague (Rinderpest)
Pernicious anemia of horses
Virus III infection of rabbits

Foot-and-mouth disease
1. Type A
2. Type O
Vesicular stomatitis of horses
Paravaccinia (No report on filtration)



Borna's disease
Fowl plague and plague of blackbirds
Guinea pig paralysis
Distemper of dogs

Trachoma and inclusion blenorrhea
Infectious papular stomatitis of cattle
Molluscum contagiosum
Warts
Contagious epithelioma (fowl-pox)
1. Chickens
2. Pigeons
Infectious myxomatosis of rabbits
Rous sarcoma of chickens
Leukemia of chickens
Lymphocystic disease of fish
Epithelioma of fish
Carp-pox
} No reports on filtration

Mumps (According to Kermorgant, a spirochetal disease)
Agalactia (According to Bridre, a bacterial disease)
Salivary gland disease of guinea pigs
Measles (rubeola)
German measles (rubella) (No report on filtration)
Grippe (influenza) (According to Olitsky and Gates a bacterial disease)
Common Colds

B
- Nairobi disease of sheep
- Catarrhal fever (blue tongue) of sheep
- African horse sickness
- Pappataci fever
- Dengue fever
- Yellow fever (According to Noguchi a spirochetal disease)

C — Rickettsid diseases
- Typhus fever
- Trench fever
- Rocky Mountain spotted fever
- Heartwater disease
- Flood fever of Japan

D — Bacterial diseases
- Oroya fever and verruga peruviana
- Pleuropneumonia
- Avian diphtheria
- Scarlet fever

Nature of Filterable Viruses.—Inasmuch as the criterion used for admission to this group is the ability to pass a fine filter, it is evident that it comprises a very heterogeneous group. Beijerinck in his early work concluded that the virus which caused the mosaic disease of tobacco was not corpuscular, but was of the nature of a self-propagating enzyme. This same idea is held and vigorously defended by many modern authorities in respect to the bacteriophage. However, the facts point to the conclusion that the viruses are corpuscular, that they multiply, pass some filters but not others, tend to settle out of solution on standing, and can be concentrated by centrifuging their solution. They are very minute, for in the light of certain evidence that we have at hand, the virus causing chicken plague, for example, must be smaller than a molecule of hemoglobin which is less than 23$\mu\mu$ to 25$\mu\mu$.

Although there is still a great deal of debate as to whether or not the viruses are living entities, all the leading authorities recognize in the viruses the characteristics of living organisms for they multiply, are susceptible to conditions which are injurious to living things, and possess the power of adapting themselves to their surroundings. Plants or animals which have been attacked by them and recover possess a high and lasting immunity; this is even more pronounced than in the case of bacterial disease. They apparently are obligate parasites, for up to the present time they can be cultivated only in living tissue. They usually have an affinity for specific cells and in the diseased animal these cells contain certain cell inclusions which are characteristic of the specific disease. The relationship of these cell inclusions to the specific disease is not understood. Some authorities consider them to be the specific causative organisms, whereas others look upon them as deteriorations of the cell due to the action of the specific virus.

The biological nature of the filterable viruses is at present largely conjecture. The fact that some possess a definite life cycle and are resistant to glycerine but susceptible to saponin and bile, points to their protozoan character. But according to Simon:

"To assume, on the basis of our present knowledge, that the filter passers are either bacteria or protozoa, is unwarranted. We may say that certain observations suggest that a few protozoa, and possibly bacteria may assume a filter-passing size, but in so far as the great majority of the filterable viruses go there is

no evidence whatsoever to warrant the claim that their taxonomic position has been established. It might possibly be best to classify them as protista, were it not for the fact that there is evidence to suggest that some of them may be inanimate even though corpuscular. Theoretically, there can certainly be no objection to the formulation of the hypothesis that disease-producing agents of corpuscular nature may exist, which in part belong to the lowest forms of life, which are neither bacteria nor protozoa and in part to the higher forms of ferments, and that the main group of filterable viruses may properly find its position here rather than among either the bacteria or protozoa."

Three of the more important diseases of man that have been shown to be due to filterable viruses are dealt with in this chapter.

SMALLPOX

Smallpox is as old as the human race and was the first communicable disease controlled by means of a vaccine. It is conveyed from man to man by the rather intimate exchange of secretions of the nose and mouth, and is due to a filter-passing agent which up to the present time has not been propagated outside the human body except in certain experimental animals. In the epithelial cells of affected areas of diseased animals appear round or oval bodies named after their discoverer, Guarnieri bodies, which vary in diameter from one to eight microns. The discoverer considered these the causative organism, but many authorities believe them to be merely cell inclusions or degenerations resulting from the action of the virus or its toxins. Similar inclusions occur in the related disease, cowpox. The ones causing cowpox occur in the cytoplasm, whereas those of smallpox occur in the nucleus. This, together with the fact that an attack of cowpox gives an immunity to smallpox and that smallpox may be transformed into cowpox, has given rise to the idea that they are two manifestations of the same disease. Smallpox virus when inoculated into cattle does not grow well at first, but eventually it becomes transformed, so that it cannot be distinguished from cowpox. Furthermore, after it is once transformed into cowpox, it cannot be changed back into smallpox.

Vaccination.—Long before the practice of vaccination was discovered, there originated somewhere in the Orient the practice of inoculation. The Chinese took scabs from smallpox patients, ground them up, and blew them into the nostrils. The Turks

inserted material from smallpox pustules into scratches made in the skin of persons to be immunized. Lady Mary Montague introduced the practice into England in 1717, and during the remainder of the century this method of inoculation was extensively resorted to in the control of smallpox. The disease thus produced was the real smallpox and could spread from one individual to another. It therefore caused an increase in the number of cases, and occasionally it had a fatal termination. However, for some reason yet unexplained the infection was less severe than that contracted in the usual manner. Through inoculation the mortality which prior to its use occasionally reached 50 per cent was reduced to less than one per cent.

It had been the belief in pastoral districts, for how long we do not know, that an attack of cowpox protected against smallpox. Benjamin Jesty, a Dorsetshire farmer, acting on this belief, rubbed some of the material from cowpox eruptions into scratches in the skin of his wife and his two infants and thus rendered them immune to smallpox. But it was Jenner who first placed vaccination upon a sound basis, when in 1796 he took pus from a cowpox pustule on the hand of Sarah Nelms and introduced it into the arm of a lad, James Phipps. Six weeks later the boy was deliberately exposed to smallpox and even inoculated with the virus. He was found to be immune, and today it is universally agreed by authorities that the control of smallpox rests upon vaccination.

Various methods have been used for the introduction of smallpox virus into the body of the individual to be immunized, such as by puncture, scarification, scratch, or incision. The method recommended by Dr. Rosenau is the scratch or incision method. A scratch about one-eighth inch long is made with a sterile needle, and a drop of the vaccine is rubbed into it. The best place to do this is on the arm, but the spot chosen must be thoroughly washed with soap and water followed with alcohol or acetone before the scratch is made.

Value of Vaccination.—The value of vaccination has been amply demonstrated. Before the days of Jenner, everyone expected to contract smallpox; now no one expects to, but the unvaccinated occasionally do. In Germany, Japan, and other places where vaccination is made compulsory and is systematically carried out, smallpox has disappeared from the native inhabitants, but in unvaccinated districts smallpox still claims its victims. In such districts it often requires an outbreak of the disease in order to force the inhabitants to avail themselves of the protection

which science offers. It is generally agreed today by authorities that the unvaccinated individual is not only endangering his own health, but he is withholding from the community a protection which rightfully belongs to it; and it is just as important in the "land of the free" that vaccination be made compulsory as is education.

Dr. Rosenau summarizes the claims made for vaccination in the following manner:

"1. 'Duly and efficiently performed it will protect the constitution from subsequent attacks of smallpox as much as that disease itself will.'

"2. It protects against smallpox for a period which varies with the individual, but which for practical purposes may be taken to average seven to ten years.

"3. The protection may be renewed by repeating the vaccination.

"4. Persons vaccinated every ten years will be reasonably protected throughout life.

"5. Vaccination and revaccination systematically and generally carried out confer complete protection to a community or nation. In other words, while individual protection is not always lasting, communal protection is absolute.

"6. A person vaccinated once and at a later time contracting smallpox as a rule has the disease in a less serious form than an unvaccinated person. The degree of favorable modification of smallpox depends upon the length of time elapsing between the vaccination and the attack of smallpox.

"7. The beneficial effects of vaccination are most pronounced in those in whom the vaccine affection has run its most typical and perfect course."

The best practice is to have children vaccinated when they are but a few months old; again when they enter school; and thereafter, at intervals of ten or more years or oftener if intimately exposed to smallpox.

Manner of Spread.—Smallpox is so infectious that it used to be taught that it was due to a "volatile virus," and consequently, spread through the air. However, authorities in general now agree that it is spread in much the same manner as other communicable diseases are. Discharges from the mouth and nose, the pus from the sores, and the scabs or scales from the skin are all known to be infectious. These may readily be transferred, either directly or indirectly from the ill to the non-immune and

cause the disease. The period of incubation is from nine to fifteen days, and the disease is communicable before the eruption occurs.

RABIES OR HYDROPHOBIA

Rabies is a disease primarily of the dog and other animals and is transmitted to man usually by a bite, although the mere licking of an abraded area of the body by a rabid animal is occasionally sufficient to transmit the disease. Pasteur proved it to be due to a virus occurring in the nervous tissue and saliva of the rabid animal. There occurs in the nervous tissue of such animals cell inclusions known as Negri bodies. These may be the actual virus or the cells injured by the virus. The Negri bodies are of importance in that they furnish a ready method by which the bacteriologist can diagnose the disease.

Symptoms of Rabies.—There is much misunderstanding concerning the symptoms of rabies. The dog in the early stages does not manifest a furious raging disposition with a tendency to bite man or other animals, nor is there a stage in the development of the disease in which the animal shows any fear of water. In the early stages it may wade into it, and lap water with relish, but it is evident this would not occur in the later stages on account of paralysis. A rabid dog manifests a condition of nervous unrest. It may travel considerable distances from its home. It attempts to hide itself, crawling under any object which may offer a temporary resting place. However, it cannot rest; it seems dissatisfied with everything. It is especially familiar with other animals, likes to be caressed by its master and if permitted will lick his hand. This is a very dangerous procedure for the virus may be in the saliva and if it gets into an abrasion is likely to cause the disease. Later the affected animal runs at an uncertain but rapid rate, and is likely to snap at anything, including man, which may cross its path. "Sooner or later the animal passes into the paralytic stage. Its lower jaw droops, the saliva accumulates in the mouth, and the animal attempts to remove it with its paws. The paralysis, beginning usually in the posterior extremities, gradually involves all the muscles of the body, and the animal dies from asphyxiation. During the paralytic stage, on account of the fact that the animal cannot close its mouth, it does not bite, but its saliva contains the virus in a large amount. Its effort to pull the thick mucus from its throat may lead a witness to believe that the animal has a bone in its throat and to

attempt to assist in its removal. Such a procedure is accompanied by great danger."

Sometimes the animal may pass directly into the paralytic stage without the early stages of restlessness; this is known as "dumb rabies." In this form the animal lies partly paralyzed with its mouth open. Man often becomes infected from handling such animals.

Transmission of Rabies.—Rabies is transferred from the lower animals to man almost wholly by the saliva which finds its way into a bite or any injured tissue. The relative danger of infection varies with the part of the body bitten and the nature of the wound inflicted. Bites on the bare surface such as the hands and face, are far more likely to give rise to rabies than are those through clothes. This is probably due to the clothing keeping back much of the virus and certain areas are richer in nerves and nearer the central nervous system, hence, the virus which follows the nerves can reach a vital point. Deep, ragged wounds, such as those inflicted by the wolf and dog, are more dangerous than the contuse wound produced by the sheep or horses.

Virus.—The active principle of rabies is a filter-passing virus which occurs principally in the central nervous system and in the saliva of the afflicted animals. It is sensitive to light, drying, and to many of the ordinary disinfectants, and is especially resistant to extreme cold. It is very sensitive to formaldehyde and nitric acid. The virus sometimes appears in the saliva as long as five days before symptoms. Therefore a good rule to follow when bitten by a dog is to watch the dog for ten days, and if the animal does not develop rabies in that time the victim of the bite is safe. If it does, he should receive the Pasteur treatment immediately.

Prevention of Rabies.—The prevention of rabies in man is easy in some districts, but comparatively hard in others, for it rests principally upon preventing it in dogs. In areas like Russia, France, and the United States where the disease is prevalent among wild animals, which in turn transmit it to the domestic animals, the problem is much greater than in England. A control of the number of dogs is essential. This is accomplished by the destruction of stray dogs and the levying of a tax on all dogs kept. Some progress has been made in the immunization of the dogs against rabies. In Japan over one hundred thousand dogs have been immunized with a great reduction in the disease. The muzzling of dogs has eradicated rabies in England. This

practice not only prevents the spread of the disease among domestic animals, but it prevents its conveyance to man and is the only effective method in a rabies-infected area.

The procedure which has reduced rabies from a comparatively frequent to a rare disease is the Pasteur treatment. This should be given in every case where individuals are bitten with rabid animals. In cases where the bite is from an apparently healthy dog it should be watched for ten days. If rabies develop in the dog during this time the patient should immediately receive the Pasteur treatment. Where the animal has been killed its head should be packed in ice and shipped to a bacteriologist prepared to make examination for Negri bodies.

MEASLES

Measles is one of the most highly communicable diseases known. In this respect it resembles smallpox. It is peculiar to man and attacks every race, both sexes, and all ages. It is often considered a child's disease, because of the fact that most individuals in temperate zones contract the disease and thus gain a life-long immunity before they reach maturity. However, it must not be forgotten that this immunization process costs annually in the United States alone approximately twelve thousand lives.

History.—We owe our first accurate description of measles to the Arabian physicians of the tenth century. Probably prior to this period it was confused with smallpox. Since then we know that it has been endemic in most districts and periodically becomes epidemic, the epidemic dying out only when there are no more non-immunes. Occasionally, it reaches virgin districts with direful results. The epidemic which swept the Fiji Islands in 1875 is an example. So far as known the disease had never touched the inhabitants of the island until that year. It was brought to them by the son of the king of the Fiji Islands, and through a conference that was held at this time, it was conveyed to all parts of the island. The population of the island numbered about 150,000; of these it is officially stated that 40,000 died of the disease. In some districts from 27 to 28 percent of the population died.

The high mortality is usually attributed to the disease being carried into a virgin population where inherited immunity is not present. The more reasonable explanation appears to be: (1)

The number ill at a given time was so great that there were not sufficient well to care for the ill. It is a well-known fact that proper nursing is the best safeguard against complications and death in measles. (2) The natives, ignorant of the disease, often bathed in streams and otherwise exposed their bodies while suffering with measles. This gave rise to dysentery and pneumonia which are prone to accompany the disease and are usually the cause of death. That this is the proper explanation appears from the fact that in certain garrisons where the natives received proper care no such mortality occurred; moreover, all epidemics among virgin populations have not shown a similar high mortality. Brownlee writes:

"I visited some years ago at Highland Glen from which measles had been absent for more than eighty years. An epidemic had just ended; it swept the glen from the western end to the eastern, hardly anyone—man, woman, or child—escaped the infection. It may be noted, by the way, in contradiction of the erroneous and quite common belief that though the soil was quite virgin in the epidemic not a single death occurred."

Probably the belief that the disease takes a more virulent form among a virgin population than among others must go the way of the other erroneous ideas namely that measles is more likely to be fatal among adults than among children. Measles often greatly disorganize armies where troops become infected, but there is no evidence that it is more fatal to adults than to children.

Virus.—Each year brings new reports of the discovery of the causative organism of measles, but whether any of the recently described organisms are the specific cause is not certain. Measles is known to be due to a filterable virus occurring in the secretions of the nose and throat. The virus is present even before symptoms appear. This accounts for the high communicability of the disease. In the early stages of the disease the virus occurs in the blood, and Anderson and Goldberger apparently communicated it to the monkey. The virus soon disappears from the blood and is probably not communicable after the fever has subsided. All evidence points to the conclusion that the scales are not infectious. The virus is frail and is soon destroyed by sunlight desiccation, disinfectants, and heat.

Manner of Spread.—The high communicability of measles early led to the idea that the disease was spread in the air, but all evidence points to the conclusion that it is spread by rather intimate contact. Any method by which the secretions of the

nose and mouth of one is quickly transferred to another favors its spread.

Immunity.—One attack of measles usually confers a lifelong immunity, although second attacks do occur. The observation of this high immunity has led within recent years to the use of a convalescent serum obtained from the individuals who have recently recovered from measles. This serum protects or else greatly mitigates the resulting cases of measles. When given in the early part of the incubation period it greatly modifies the severity of the disease, and the result is a lasting immunity; consequently, some authorities are recommending this procedure.

Prevention.—Quarantine, the closing of schools, and the use of terminal fumigation, all appear to be without effect on the spread of measles; consequently, the only thing that can be done is to use those well-established means of preventing complications and sequels. The care of the eyes during and just after an attack can do much in saving eye sight. Darkened rooms during the attack and no attempt to use the eyes too soon after a case of measles are good precautions. Measles predispose to pneumonia and tuberculosis; consequently, the convalescent should avoid individuals ill of these diseases, as well as all those conditions which contribute to them. Statistics indicate that the main mortality from measles occurs among the young, and that 90 per cent of the fatal cases occur during the first five years of life; consequently, when parents intentionally, or carelessly expose their children to the disease with the belief that the younger children have it in a light form, they are making a grave mistake. Furthermore, it should be remembered that the infectious diseases take not only the weaklings, but often the most vigorous and robust. This is well pointed out by Vaughan in the following passage:

"Even the great philosopher of modern science, Herbert Spencer, taught that infectious diseases, especially among children, were in the long run beneficial since they cut out in early life those who are unfit and give greater opportunity for the proper development of those who should live. Is there any basis in fact for this belief? Did smallpox during the centuries when it was uncontrolled by vaccination kill only weaklings and spare the robust? Do measles, scarlet fever, whooping cough, infantile diarrhea, play that function today? If these questions can be answered authoritatively in the affirmative, then we should hesitate in our attempt to eradicate the infectious diseases,

especially those most prevalent and most fatal in infancy and childhood. No child acquires measles, whooping cough, or scarlet fever, unless exposed to the infection. The danger to the life of the individual child depends upon many conditions, especially upon the state of health at the time of the exposure and the amount and virulence of the infection transferred to the child at the time of exposure. Are these conditions determined or modified by the degree of fitness or unfitness to live possessed by the individual child? If a careless nurse carries articles of clothing soiled with discharges from a scarlet fever patient into a household where there are children, is there any reason for believing that the less fit of these children are more likely to come in contact, or are more likely to suffer from coming in contact, with the imported virus? An individual with open tuberculosis deposits bacilli-laden sputum on the floor of a school-room filled with children in all degrees of physical fitness and unfitness. By what law or by what chance will the less fit of these children be infected, while the more fit escape? Badly infected milk may be used in a children's school. Some will drink more of the poisonous milk and some will drink less, and possibly a few will drink none of it. All will suffer or escape as they fall into one of these classes. What will lead all the unfit children to drink the poisonous milk and what will keep all the fit children from drinking any of it? If the fitness of a child to live is to be determined by passing through some such ordeal as exposure to a highly dangerous infectious disease, let us not leave this to nature but proceed to inoculate every child in a scientific and exact way. Suppose that we determine the minimum amount of white arsenic that may kill, say a weakling at one year of age; then on its first birthday we administer this dose of arsenic to every child. Are we to suppose that all those who might grow into inefficient or vicious citizens would die, while all those who are to develop into perfect manhood or womanhood are to live? If we are going to imitate nature in this experiment we must not weigh out the dose given each child, but measure it out, guess at it; and if we are going to imitate nature more accurately we shall select a blind person to measure out for each child its dose. The idea that the infectious diseases of childhood kill only the weaklings, or even kill the weaklings in larger proportion, is based upon a complete misunderstanding and misinterpretation of all epidemiological science. It would be just as sensible to attempt to improve the race by mustering all the children in a community

and have them pass down a street while blind-folded men with repeating rifles fired into the crowd. Fortunately, even the preachment of Herbert Spencer on this point has in no way abated or hindered the attempts of the sanitarian to control and eradicate the infectious diseases prevailing at any period of life."

Every known means should be used in the prevention of communicable diseases, and it is well to constantly have in mind that the fundamentals on which prevention is founded are: (1) Communicable diseases are due to microörganisms, and these are the descendants of other similar microörganisms. (2) The great majority of microbes which cause disease in man multiply only in the body of man and the lower animals. (3) The overwhelming majority of all diseases are transmitted by direct contact. Their carriers are fingers, flies, and food. (4) A high state of bodily health should be the aim of all, but it does not necessarily confer immunity to the communicable diseases. (5) In some diseases immunity may be acquired by a mild attack of the disease produced by vaccination; in others it may be transferred from one animal to another through the blood by means of so-called antitoxins.

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